Monitoring deep-sea benthic biodiversity using environmental DNA approaches to compare trawled and untrawled sites

by

Delene Oosthuizen

Thesis presented in fulfilment of the requirements for the degree of Master of Zoology in the Faculty of Science at Stellenbosch University

Supervisor: Prof Sophie Von der Heyden

Co-supervisor: Dr Mat Seymour

December 2021

Declaration:

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December 2021

Abstract:

The deep-sea is the largest environment on earth and yet it remains understudied. Environmental DNA (eDNA) metabarcoding provides a cost and time effective method to characterise and study deep-sea benthic communities. This study is the first study in South Africa to use eDNA to study benthic communities in the deep-sea. Sediment samples were collected from 29 sites on Childs Bank off the West Coast of South Africa. Certain sites had been closed to trawling for 5 years while others had been left open to trawling. DNA was extracted from the sediment samples and used to determine the taxonomic composition of the benthic communities. When compared to existing species inventories from the area, it was found that the eDNA metabarcoding recovered similar numbers of taxa, some of which were not listed in other species lists. However, many taxa could only be assigned to higher taxonomic levels such as order as many species are not represented in barcode databases. Environmental data such as site depth and sediment type and composition were also collected from the study sites. Community composition was then compared between sites to determine whether trawling, depth or sediment type affected community composition. Contrary to what was expected, depth was the only factor with a significant effect on community composition.

Opsomming:

Die diepsee is die grootste habitat op aarde maar is steeds tot ‘n groot mate nog min bestudeer. Omgewings DNS-kodering bied ‘n tyd en koste effektiewe manier om diepsee organismes te ondersoek. Hierdie studie is die eerste van sy soort in Suid-Afrika om oDNS te gebruik om bentiese gemeenskappe in en op die diepsee bodem te bestudeer. Sediment monsters is van 29 data versamelpunte op Childs Bank langs die Weskus geneem. Dele van die area is gesluit vir treilvisserye terwyl ander dele oopgelos is. DNS is van die sediment monsters geïsoleer en gebruik om die taksonomiese samestelling van bentiese gemeenskappe te identifiseer. In vergelyking met ander spesie opnames van die area, het die studie soortgelyke getalle taksa gevind, waarvan sommige nie op die ander lyste teenwoordig was nie. ‘n Gedeelte van die taksa kon nie tot laer taksonomiese vlakke geïdentifiseer word nie omdat baie van hierdie spesies nie in DNS-kodering databasisse teenwoordig is nie. Ander inligting uit die omgewing (nl. diepte en sediment-tipe) is ook versamel. Die bentiese gemeenskap samestellings tussen areas kon vergelyk word om te bepaal of diepte, sediment-tipe of blootstelling aan treilvissery ‘n invloed op die samestelling van gemeenskappe het. In

teenstelling met verwagtings, het net diepte ‘n noemenswaardige effek op bentiese gemeenskap samestellings getoon.

Acknowledgements:

I would like to acknowledge the following people and institutions without whom this project would not have been possible: NRF for funding, Dr Lara Atkinson from SAEON for providing samples, Molly Czachur for great help in the lab, my family for all their ongoing support and finally my supervisors, Prof Sophie von der Heyden and Dr Mat Seymour for their time and patience.

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General background

Marine biodiversity in the deep-sea

Biodiversity, the variation of life in terms of species, genetics and functional traits (Cardinale et al. 2012), is a critical component of ecosystem functions and services. Anthropogenic effects account for the majority of the ongoing decline in biodiversity, which has significant impacts on natural ecosystems, human well-being and global economy (Cardinale et al. 2012; Pecl et al. 2017). There is increasing concern about the rapid decline in biodiversity, resulting in several attempts to establish conservation policies for maintaining or improving biodiversity (Cardinale et al. 2012). However, international biodiversity conservation targets are not adequately met in terms of sufficiency, i.e., how much should be protected, or efficiency, i.e., allocating conservation resources efficiently (Di Marco et al. 2016). Even with the targets that are being met, as in the case of increasing public awareness of the biodiversity crisis and increasing protected area coverage, there is still concern that there may be a delay before the effects result in positive changes in biodiversity conservation and recovery (Tittensor et al. 2014; Di Marco et al. 2016).

Marine systems are the largest ecosystems on earth. It is estimated that, of the ~8.7 million species known to science, 'known' referring to species that have been named and described, ~2.2 million are marine species (Mora et al. 2011). Marine ecosystems provide several provisioning and regulating ecosystem services, through food and mineral resources, nutrient cycling, and climate regulation (Worm et al. 2006; Barbier 2011; Pörtner et al. 2014). However, placing economic or monetary value on these services remains difficult because of the interconnectedness of different marine and coastal systems (Barbier 2011). Marine ecosystems face a variety of anthropogenic threats such as overexploitation, pollution and mining for natural resources, as well as threats from global climate change, including rising surface temperatures and increased ocean acidification (Costello et al. 2010; Pörtner et al. 2014). Despite the importance and the threats to marine biodiversity, perhaps as many as 70-90% of marine species remain undescribed (Costello et al. 2010; Mora et al. 2011) although later estimates revised these values to 24-31% (Costello et al. 2012). Many of these unknown species are thought to be rare, cryptic, and small and/or have restricted ranges, as there is a taxonomic bias towards describing large, widespread and commercially important species (Mora et al. 2011; Costello and Chaudhary 2017). In addition, many ocean habitats are

remote and hard to access and sample, which also makes sampling more difficult. This is particularly true for the deep-sea, which remains highly understudied (Mengerink et al. 2014; Laroche et al. 2020).

The deep-sea is defined as the water column and seafloor deeper than 200m and represents the largest environment on earth, covering about 60% of the planet's surface (Glover and Smith 2003; Thurber et al. 2014; Costello and Chaudhary 2017). Deep-sea habitats remain especially understudied, mostly because they are more difficult and costly to sample, driven largely by their remoteness (Benn et al. 2010; Costello et al. 2010; Thurber et al. 2014). The seafloor has presumed high levels of biodiversity (Levin et al. 2001; Armstrong et al. 2012; Thurber et al. 2014; Sinniger et al. 2016; Laroche et al. 2020) of which many species are yet undiscovered (Mengerink et al. 2014; Thurber et al. 2014; Sinniger et al. 2016), particularly meiofaunal taxa (Sinniger et al. 2016; Laroche et al. 2020).

The majority of deep-sea taxa are benthic, including both epifauna and infauna, organisms living inside the sediment and on top of the sediment, respectively (Miller and Wheeler 2012). In addition, there are various fish species utilising both benthic and mid-water environments. Benthic fauna are usually divided into size categories – megafauna (>>1mm), macrofauna (>1mm), meiofauna (0.1-1mm) and microfauna (<0.1mm) (Miller and Wheeler 2012). Although numerous deep-sea species have been described, ecological information about them often remains unknown, including their distribution ranges, life history characteristics and dispersal abilities, population diversity and connectivity, and demographic parameters (Baco et al. 2016; Taylor and Roterman 2017), as well as their functional diversity. As with pelagic and coastal marine systems, abiotic variation in deep-sea habitats such as sediment properties, nutrient availability and oxygen availability, depth as well as features of the seafloor play a role in shaping the distribution and population structure of deep-sea benthic species (Levin et al. 2001; Etter et al. 2005; Jennings et al. 2013; Baco et al. 2016; Taylor and Roterman 2017). Certain deep-sea environments such as hydrothermal vents and seeps have unique chemical properties and, as such, have high numbers of endemic species (Ramirez-Llodra et al. 2011; Thurber et al. 2014; Cowart et al. 2020). Other structures, such as seamounts, play a role in the temporary aggregation of species such as breeding/spawning events of fish species (Norse et al. 2012). Seamounts may also act as biodiversity hotspots, refugia for species that are impacted by disturbance in their

environment and serve as stepping stones for dispersal of species (Laroche et al. 2020). In many cases, species make changes to their environment, forming biogenic habitats, many of which are important habitats for other species, such as cold coral reefs acting as nurseries for some fish species (Thurber et al. 2014). It is thought that, as methods of mapping and studying the seafloor improve, that scientists will find that it is a more heterogeneous environment than previously thought and that many of the features of heterogeneity are on a very fine scale (Danovaro et al. 2014). Although the above features add heterogeneity to the deep-sea, it is still largely a more homogenous environment when compared to other marine systems, with ~ 90% of the seafloor characterised by continental slope and abyssal 'mud plains' (Glover and Smith 2003; Laroche et al. 2020).

Importantly, the combination of physical and abiotic environmental variation make for complex metacommunity dynamics. Previously it was assumed that deep-sea species have higher dispersal capacity and thus are more connected than species from shallower environments (Baco et al. 2016). However, when population studies from a number of different deep-sea species from different habitats were compared, it was found that the levels of connectivity and dispersal across a range of taxa and habitats are similar to those in shallower environments, even though it differed between deep-sea species from different taxa and habitats and with different lifestyles and larval types (Baco et al. 2016). However, knowledge about population structure and diversity are mostly lacking, as is genetic information about many deep-sea species (Taylor and Roterman 2017). This information is necessary in describing conservation units as well as the design of marine protected areas (Taylor and Roterman 2017).

The deep-sea provides many important ecological and economic functions and services, including nutrient cycling, sequestering atmospheric CO_2, waste absorption, oil and gas reserves, as well as supporting both vertebrate and invertebrate fisheries (Dallagnolo et al. 2009; Armstrong et al. 2012; Norse et al. 2012; Mengerink et al. 2014; Thurber et al. 2014). The deep-sea is also considered a source of genetic resources, which are a range of biological material such as genes, proteins and naturally produced chemicals which have or potentially have economic value as natural products (Harden-Davies 2017). Given the lack of knowledge on ecological and functional diversity of many deep-sea species, it is difficult to place value on the services it provides (Armstrong et al. 2012). This is problematic, as pressures on the

deep-sea keep increasing as human populations grow and the demand for resources increases (Armstrong et al. 2012). Although it is difficult to monitor impacts, given the remoteness and difficulties of sampling deeper areas, there have been some recorded changes. This includes the effects of global climate change on changes in temperature, acidification of the water column associated with an increase in CO_2, the possible release of methane from seeps and an increase in hypoxic zones (Ramirez-Llodra et al. 2011) as well as the movement of species into deeper areas, affecting the native benthic species (Levin and Le Bris 2015).

In terms of direct human related impacts and changes, the deep-sea is used to dump different waste products, including radioactive substances, munitions and sewage, laying of telecommunications cables and gathering of resources like fish, gas and oil (Benn et al. 2010; Ramirez-Llodra et al. 2011; Mengerink et al. 2014; Thurber et al. 2014). The impacts of these activities vary, but in combination affect a large part of the ocean floor. Some of the greatest concerns are pollution, as many waste substances that are dumped in the deep-sea are toxic to the species living there, and habitat loss due to chemical pollution, physical destruction from trawling gear, mining activities and dumping of large structures (Ramirez-Llodra et al. 2011). Overexploitation of resources, including the use of trawling, also remains one of the greatest threats to the deep sea (Ramirez-Llodra et al. 2011), especially with regards to fisheries. Global fisheries have been increasing the depth at which they catch by up to 62.5 m decade^{-1} since the 1950s (Watson and Morato 2013). In addition to an increase in fishing depth, there is also a global decline in the trophic level of species caught (Pauly et al. 1998). As the number of higher trophic level species such as predatory fish decline, fisheries turn to species at lower trophic levels like planktivorous fish and invertebrates (Pauly et al. 1998). Overfishing lower trophic species has a number of ecological impacts such as the simplification of food webs causing instability and trophic cascades contributing to algal blooms (Pauly et al. 2002). Deep-sea fisheries also have an impact on benthic communities, both by causing physical damage such as sediment resuspension and seabed destruction by heavy gear, and by causing changes in biodiversity, community composition and species abundances leading to changes in the system (Clark et al. 2015). Trawling causes physical damage to the sea floor, breaking habitat forming species such as corals, which may take many years to grow back, disrupting sediments and causing erosion of the seafloor and homogenising the surface topology (Ramirez-Llodra et al. 2011; Clark et al. 2015). Further, by disrupting the sediment of the seafloor, the composition of benthic communities is also

impacted (Kaiser et al. 2006; Atkinson et al. 2011a; Clark et al. 2015). Deep-sea fishing vessels are large and are able to remove many individuals of their target species from the ecosystem at a time, potentially leaving populations with little resilience to the impacts of large-scale fishing as they are removed faster than they are able to reproduce (Norse et al. 2012). Many of these important fisheries species are slow-growing and long-lived species that are also often slow to mature, adding to their vulnerability to overexploitation, as well as climate change (Morato et al. 2006; Ramirez-Llodra et al. 2011; Norse et al. 2012; Levin and Le Bris 2015). With the increasing extent and depth of fishing, as well as ecological implications of fishing down the food web alongside the global increase of demand for fish, deep-sea fisheries are becoming more unsustainable (Pauly et al. 2002; Norse et al. 2012). In all these issues, the need for adequate conservation practices is critical (Robison 2009). As large areas of the deep-sea fall outside the boundaries of specific states, it is difficult to establish effective governance and conservation practices, both in terms of resources as well as biodiversity (Harden-Davies 2017).

Environmental DNA

With all the threats and pressures acting on coastal, pelagic and deep-sea environments, it is crucial to gain better understanding of their biodiversity and its distribution, as this can go towards supporting management decisions (Mengerink et al. 2014; Thurber et al. 2014; Laroche et al. 2020). Novel molecular tools such as metabarcoding from environmental samples are becoming a promising tool to rapidly survey the environment for multiple species at a time (Creer et al. 2016; Deiner et al. 2017). In marine systems, the use of molecular tools can provide new insights into species diversity and distribution as well as being a useful tool for long-term monitoring of biodiversity and indicators of ecosystem stress (Goodwin et al. 2017).

Organisms shed genetic material in various forms, such as skin, hair, faeces, blood, and saliva, resulting in intra- and extracellular DNA accumulating in the environment (Bohmann et al. 2014; Rees et al. 2014). Extracellular DNA here refers to cases where cell material has broken down but the DNA molecule is still intact. Environmental DNA can be filtered and extracted directly from an environmental sample (e.g. water, sediment or air) and used to answer various ecological questions (Thomsen and Willerslev 2015; Creer et al. 2016; Deiner et al. 2017). DNA in the environment can also be used to study historical patterns of

biodiversity when sampled from environments such as ice or sediment cores and permafrost and is then referred to as ancient DNA (aDNA) (Thomsen and Willerslev 2015; Deiner et al. 2017).

Environmental DNA is extremely versatile and can be collected from different types of environments. In terrestrial systems, eDNA samples have been collected from soil (Drummond et al. 2015), air (Kraaijeveld et al. 2015), faeces (Zhu et al. 2011) and even from blood that leeches have consumed (Schnell et al. 2012). Aquatic eDNA samples (both from freshwater and marine systems) can be collected from either water or sediment samples (Deiner et al. 2017). DNA can also be collected from ice cores, as in the case of aDNA studies (Thomsen and Willerslev 2015). Collecting these samples varies depending on the environment, for example, water samples could be collected by scooping water into a bottle and then filtering it through a microfiber filter (e.g. Yamamoto et al. 2016), while a soil or sediment sample could be collected with cores (e.g. Sinniger et al. 2016; Nascimento et al. 2018). Regardless of the physical sampling method chosen, it is essential that the sampling strategy is truly representative of the study area and the chosen community in order to ensure that as many of the taxa as possible can be identified (Creer et al. 2016; Deiner et al. 2017). Replication is also important in later analytical steps as the number of replicates chosen, during sampling, extraction and during library preparation, have been shown to impact the number of taxa recovered (Ficetola et al. 2015; Yamamoto et al. 2017). Sample volume has also been shown to influence the biodiversity estimates from eDNA sample in sediment samples to some degree (Nascimento et al. 2018), while studies have successfully used a variety of different volumes of water when collecting eDNA in the water column (Deiner et al. 2015; Mächler et al. 2016; Yamamoto et al. 2016; Lear et al. 2018). The origin, state, transport and fate of eDNA also vary between different environments and may significantly affect the taxa identified (Barnes and Turner 2016; Deiner et al. 2017). DNA in the water column usually degrades within a few weeks or even days and is used to get a snapshot of what species are physically present or were present recently (Moushomi et al. 2019). In contrast, DNA in aquatic sediments, generally degrades more slowly due to the more anoxic conditions reducing nuclease degradation, allowing DNA fragments to persist longer (Corinaldesi et al., 2011; Thomsen and Willerslev 2015; Turner et al. 2015). DNA is also more concentrated in sediments than in the water column (Turner et al. 2015; Holman et al. 2019) and deep-sea sediments have been called the ‘largest reservoir of DNA in the world

oceans' (Dell'Anno and Danovaro 2005). The higher concentration of DNA in sediments is thought to be a result of DNA molecules being protected from nuclease degradation when they are adsorbed onto the sediment matrix (Torti et al. 2015), the number of taxa living on and in the seafloor (Torti et al. 2015) and DNA that settles from the water column to the seafloor (Turner et al. 2015). Overall, the 'lifetime' and transport of eDNA is affected by several environmental factors including UV radiation, salinity, pH, substrate absorption, ocean currents, tidal fluctuations, dilution, etc., creating some difficulties in the interpretation of eDNA results (Deiner et al. 2017), particularly across different habitat types.

Once the chosen environmental sample has been collected, DNA can be extracted using a variety of methods, again depending on the sample and chosen protocol (Lear et al. 2018). Commercial kits such as Qiagen's Powersoil kits, are regularly used and recommended (Lear et al. 2018), although users may alter some steps to suit the needs and specifics of the samples collected. The resulting extracts are then amplified, often via a two-step PCR process (Deiner et al. 2017). Specific primers can be chosen or developed depending on what taxonomic group is chosen e.g. a primerset specific to fish (e.g. Miya et al. 2015), or decapod-specific primers (Komai et al. 2019) or even to a specific species (e.g. Jerde et al. 2011). On the other hand, more universal primers can be chosen if the aim is to identify which taxa make up the community (e.g. Stoeck et al. 2010). The PCR products are eventually sequenced on an appropriate high throughput sequencing (HTS) platform and then processed via various bioinformatic pipelines (Deiner et al. 2017; Bani et al. 2020).

The successful applications of environmental DNA in studying biodiversity are broad. It is commonly used to monitor the presence or absence of single species such as rare, endangered, cryptic or invasive species (e.g. Ficetola et al. 2008; Jerde et al. 2011; Thomsen et al. 2012a; Schnell et al. 2012; Biggs et al. 2015; Boussarie et al. 2018). More recently, environmental DNA has been used to study population dynamics (Sigsgaard et al. 2016; Stat et al. 2017). However, multiple studies have also sampled entire communities to determine species composition of an area and distribution patterns (e.g. Fonseca et al. 2014; Drummond et al. 2015; Yamamoto et al. 2016 & 2017), as well as to observe the impacts of human induced change such as urbanisation and pollution, as well as infrastructure, on community structure and diversity (see Lejzerowicz et al. 2015; Kelly et al. 2016; Laroche et al. 2017; Xie et al. 2017). Importantly for the context of this study, eDNA has been used effectively on

deep-sea sediment samples to gain insight into benthic meiofaunal diversity and distribution patterns (Fonseca et al. 2014; Sinniger et al. 2016; Zhao and Xu 2016; Laroche et al. 2020; Cowart et al. 2020).

When compared to more conventional methods of surveying biodiversity such as physically capturing or counting specimens, eDNA metabarcoding is often more efficient in terms of sampling effort and time (Bohmann et al. 2014; Deiner et al. 2017). Camera traps, trawl grabs and other physical survey methods can only capture a small part of all the organisms in the environment at a specific time, while an eDNA sample can usually provide a broader assessment with less capacity and effort, including larger species which are able to avoid equipment such as camera traps or trawl nets or even just the presence of humans in their environment (Thomsen et al. 2016; Boussarie et al. 2018). It is also non-invasive and does not cause distress to the individuals being studied (Thomsen et al. 2012a; Schnell et al. 2012). In terms of accuracy, a number of studies have compared eDNA techniques to more traditional sampling methods. The results from these studies generally show that the different methods are comparable (Lejzerowicz et al. 2015; Thomsen et al. 2016; Yamamoto et al. 2017). For example, when Yamamoto et al. (2017) compared traditional sampling methods (14 years of underwater visual censuses) of fish species in Maizuru Bay, Japan, to eDNA metabarcoding, they not only found more than half the species found in 14 years of traditional sampling in about six hours of eDNA sampling, but also detected species that underwater censuses missed. These species occurred only rarely in the bay and even then probably only as pelagic larvae (Yamamoto et al. 2017). When comparing morphological data and molecular data from sediment eDNA samples, Lejzerowicz et al. (2015) showed that both methods performed equally well when testing the impacts of fish farms on the benthic community in the area.

While using eDNA approaches to study biodiversity offers many exciting possibilities, it is worth noting that it has certain limitations. When comparing eDNA datasets to species inventories of an area, there is often not a complete overlap of taxa found, as mentioned above. This can be a result of certain species being able to avoid camera traps or trawl nets or simply just being a very rare in the environment (Schnell et al. 2012; Thomsen et al. 2016; Yamamoto et al. 2017; Boussarie et al. 2018). In such cases, eDNA samples are able to complement and add to already known taxonomic datasets. In other cases, eDNA samples

may miss species that are known to be present in the study area or are found to be less accurate than other methods (e.g. Foote et al. 2012). Some reasons for this are: a) that the eDNA concentration of these species at the site was too low to be detected at the time of sampling; b) if their eDNA was present in sufficiently high concentrations, the resulting sequences may not have been identified or present in the reference database or c) eDNA may have been present but was not captured by the sampling methods used or d) methodological issues such as primer mismatches (Cowart et al. 2018).

There have also been various attempts to elucidate whether eDNA samples can be used to make inferences about species abundances and not only their presence or absence (Kelly et al. 2014; Tillotson et al. 2018). This has mainly been tested in closed environments such as aquaria with known species abundances (Thomsen et al. 2012a; Kelly et al. 2014). A few studies also tested this in natural freshwater (Lacoursière-Roussel et al. 2016; Tillotson et al. 2018) and marine systems (Thomsen et al. 2016; Yamamoto et al. 2016). It has been found that eDNA concentration may be correlated with relative abundance of individuals (Thomsen et al. 2012a; Kelly et al. 2014; Yamamoto et al. 2016), although it remains uncertain to what extent eDNA concentration can be used to infer species abundances as it may be confounded by the rate of eDNA production, transport and how long it persists in the environment, as these factors often differ between taxa and different environments (Deiner et al. 2017). Bista et al. (2018) showed that metabarcoding - when using a single amplicon - does not always provide accurate information regarding species biomass. When the results for multiple amplicons were combined, the result was improved (Bista et al. 2018). Additional caveats to inferring abundance occur in the laboratory where primer bias and subsampling could drive the loss of rare reads, causing increased variance in the abundance of the reads observed as well as losing rare reads (Deiner et al. 2017). As such it remains important to use eDNA as a complement to other tools when studying biodiversity.

Another important aspect of using environmental DNA as a tool to monitor biodiversity is validating the results with available biodiversity inventories, such as reference databases and/or species lists from an area. Reads from an eDNA sample can be clustered into operational taxonomic units (OTUs), based on a fixed sequence dissimilarity threshold (often 3%) (Callahan et al. 2017), which are then compared to existing genetic/barcoding databases in order to identify taxa (Creer et al. 2016; Deiner et al. 2017). Importantly, however, OTUs

are not always equal to species (Carugati et al. 2015). In many cases, OTUs can only be identified to higher taxonomic levels such as family (Carugati et al. 2015). More recently, methods have been developed that can determine exact amplicon variants (ASVs) that can distinguish sequence variants that differ by as little as one nucleotide which allows for very fine scale resolution of a dataset (Callahan et al. 2017). OTUs and ASVs can then be assigned a taxonomic identity by comparing them to existing sequences in reference databases (e.g. BOLD, NCBI, Silva, Greengenes). The taxa identified can then be cross checked with existing species lists to confirm the validity of the dataset, as well as to compare the efficiency of different sampling methods. However, taxonomic identification is only viable where existing reference databases allow assignment of molecular sequences of interest and where the sequences recovered can be used to identify a low enough taxonomic level (Carugati et al. 2015; Sinniger et al. 2016; Stat et al. 2017). In other words, taxa can only be identified as far as the reference database is complete (Creer et al. 2016; Deiner et al. 2017).

Very few genetic studies have been conducted in the deep-sea and as a result there is a very limited molecular database to use for taxonomic assignments, particularly for less well studied taxa like small benthic invertebrates (Sinniger et al. 2016; Taylor and Roterman 2017; Laroche et al. 2020). Some studies circumvent this problem by collecting specimens alongside their eDNA samples and can create custom barcoding databases to compare their OTUs to (e.g. Hänfling et al. 2016). Unfortunately, this is not always a feasible option as many environments may be hard or costly to sample.

South Africa's marine realm

The South African coastline spans ~3650km and has an Exclusive Economic Zone (EEZ) of about 1 million km^2 (Griffiths et al. 2010). The coast is characterised by the cold Benguela current and the Atlantic Ocean on the West and the warmer Agulhas current and the Indian Ocean on the East, leading to differences in temperature, upwelling regimes and productivity (Griffiths et al. 2010). These differences also lead to differences in species richness, with the West Coast being less species rich than the East coast (Awad et al. 2002; Griffiths et al. 2010). South Africa is globally known as a region with high biodiversity, both in terrestrial and marine systems (Griffiths et al. 2010). When compared to many other African countries, South Africa's marine biodiversity has been relatively well documented. Over 12,000 marine species have been described, although this number is expected to increase as new species are

described and existing taxonomy is revised (Griffiths et al. 2010; von der Heyden 2011). However, numerous questions about the marine biodiversity of South Africa still remain, as there is a bias towards sampling larger and more commercially valuable species instead of smaller, less valuable species. In addition, sampling and species descriptions have been focused on intertidal and shallower coastal habitats, where most taxa, including invertebrates and algae as well as larger vertebrates have been described (Griffiths et al. 2010). Similarly, the pelagic offshore environment has also received a lot of attention, especially in terms of accounting for commercially important fishes as well as marine mammals (Griffiths et al. 2010). Many small bodied invertebrate taxa, such as Nematoda and Plathyhelminthes, are thought to be underrepresented although other groups, such as Mollusca and Arthropoda, have been relatively well studied (Griffiths et al. 2010). In contrast to the shallower coastal systems, the deep-sea, and especially the benthic deep-sea environment, has received little attention, particularly the deeper areas, with 83% of deep-sea samples taken from sites less than 100m deep (Griffiths et al. 2010). More recently, projects have been set up to sample South Africa's deep-sea and a field guide to numerous deep-sea benthic invertebrates in South African waters was recently published (Atkinson and Sink 2018). However, little is known about the ecology, functional roles and life histories of many deep-sea inhabitants.

As is the case with global marine biodiversity, South African marine environments also face multiple threats. Some are directly linked to anthropogenic activities such as the introduction of alien species, exploitation from fisheries, the pollution and destruction of natural habitats, increasing coastal developments and mining (Sink et al. 2012b; Mead et al. 2013). Other threats include environmental changes that are indirectly linked to anthropogenic climate change for example, changes in temperature and upwelling regimes (Mead et al. 2013). Various marine protected areas (MPAs) have been established to protect South African marine biodiversity but until recently there were no offshore/deep water MPAs in South Africa (Sink et al. 2012b). Twenty-two new offshore MPAs were proposed as part of Operation Phakisa, an initiative of the government to promote maritime activities and make use of the economic potential of the country's marine environments (Harris et al. 2014; Sink 2016) but have only recently been officially implemented (Sink et al. 2019). One of the greatest challenges facing decisions surrounding conservation management is, especially in relation to measuring ecological changes resulting from anthropogenic activities, that there is little or no baseline data from pristine or near-pristine environments to measure change

against (Currie et al. 2020). The value of such a dataset was illustrated by Currie et al. (2020), when they were able to replicate historical exploration surveys along the Agulhas Bank off the South African coast before trawling was commercially important. The authors replicated all aspects of the historical survey (trawl gear, speed, depth, site, etc.) and re-surveyed three sites along South Africa's important inshore trawling areas. They compared catch assemblages and found that catch composition had changed significantly over a period of 111 years, with certain species that had once dominated catches being almost completely absent and species that were rare now dominating catches. This indicates that benthic habitats may have been altered by a long history of trawling and that fish communities may have changed as a result (Currie et al. 2020).

Fisheries are an important part of South Africa's economy, worth about over R7 billion per annum (DAFF 2014; de Moor et al. 2015). Some of the most commercially valuable fisheries are situated on the West Coast (Griffiths et al. 2010; DAFF 2016). The Benguela current flows northward along this coast, with intense seasonal upwelling that contributes to making the West Coast a highly productive system (Griffiths et al. 2010; Atkinson et al. 2011a). This system supports diverse benthic, demersal and pelagic fisheries, including species such as the Cape hakes (*Merluccius capensis* and *M. paradoxus*) and kingklip (*Genypterus capensis*) (Atkinson 2009; Griffiths et al. 2010).

South African fisheries make use of a number of different methods depending on whether the target species are benthic or pelagic. Pelagic fish species are often caught by methods such as purse-seine nets, while benthic or demersal species are caught by trawling. Trawling for commercially exploited species, both inshore and offshore, occurs along most of the South African coastline (Sink et al. 2012a), with twenty-seven marine habitats identified in the South African trawl footprint (Sink et al. 2012a). Of these, nine habitats have been identified as areas of concern based on multiple criteria related to trawling extent and vulnerability and as such are priorities for management and conservation (Sink et al. 2012a). These include the Southern Benguela Canyon, Southern Benguela Muddy Shelf Edge, Southern Benguela Hard Shelf Edge, Agulhas Canyon, Southern Benguela Gravel Shelf Edge, Agulhas Gravel Outer Shelf, Southern Benguela Gravel Outer Shelf, Southern Benguela Submarine Bank, Southern Benguela Sandy Shelf Edge (Sink et al. 2012a).

The Benguela system off the West Coast of South Africa has been commercially trawled since the early 1900s (Atkinson 2009; Sink et al. 2012a). In many parts of the world trawling is recognised to have numerous impacts on species, both those that are physically caught and those that are affected by the physical changes to the habitat caused by trawling equipment (e.g. de Juan et al. 2007; Atkinson 2009; Ramirez-Llodra et al. 2011; Cook et al. 2013; Fleddum et al. 2013; Clark 2015). However, very few studies have attempted to assess the impacts of trawling on marine biodiversity in South Africa (Sink et al. 2012a; Fleddum et al 2013). One of the few studies to date that described the effects of trawling on diversity in this area, Atkinson et al. (2011a), assessed the impact of trawling intensity on benthic infauna and epifauna in this area by comparing heavily and lightly trawled sites along the West Coast of South Africa. They found that infaunal abundance, biomass and species richness were not significantly impacted by trawling, contrary to the findings of similar studies in other parts of the world (Jennings et al. 2001; Hinz et al. 2009; Atkinson et al. 2011a), although Clark et al. (2015) reported similar effects on infaunal species in other parts of the world. This difference in impacts may be a result of the fact that the maximum annual trawling intensity is less in South Africa or that the sampling depth in this study was deeper (Atkinson et al. 2011a; Fleddum et al. 2013). On the other hand, Atkinson et al. (2011a) also found that the epifauna of these sites showed significant declines in abundance, species richness and diversity (Atkinson et al. 2011a). A follow-up study by Fleddum et al. (2013) used biological traits analysis (BTA) to examine the differences in traits of benthic communities between heavily and lightly trawled sites along the West Coast of South Africa, using the data from Atkinson et al. (2011a). Their results showed that both infaunal and epifaunal organisms showed significant differences in traits between highly and lightly trawled areas (Fleddum et al. 2013). A higher percentage of epifaunal traits showed significant differences, indicating that demersal trawling may have a greater impact on epifaunal species, supporting the findings of Atkinson et al. (2011a) (Fleddum et al. 2013).

Despite the uncertainty regarding the impact of trawling on deep-sea benthic marine ecosystems in South Africa, trawling is still recognised as a threat to South African deep-sea benthic habitats, yet without a good overview of species diversity, it is difficult to gauge impacts and provide support to future management and conservation decisions (Atkinson et al. 2011a; Sink et al. 2012a).

As part of an ongoing project with the South African Earth Observation Network (SAEON) on assessing the potential recovery of benthic habitats after trawling, three trawling lanes just west of Childs Bank were closed for trawling in January 2014, while two others were left open to trawling. Since then, the focus has been to characterise fish and invertebrate (both epifaunal and infaunal species) diversity, as well as analysing sediment properties to determine the effects of trawling on this region. To do so, both grab samples and benthic camera images were used to identify species and comparisons between sites. Childs Bank is an offshore submarine feature off Hondeklip Bay on the West Coast of South Africa (Sink et al. 2012a) (Figure 1) and falls under one of the nine endangered offshore habitats, the Southern Benguela Submarine Bank (Sink et al. 2012a). It is also part of one of the new MPAs of Operation Phakisa, as it is considered vulnerable to mining and trawling as well as being identified as an important area for supporting bycatch management and fisheries sustainability (Harris et al. 2014a).

Research aims

Marine biodiversity is understudied and a large extent of it remains unknown (Costello et al. 2010), especially in the deep-sea (Sinniger et al. 2016), and is also highly threatened and impacted (Costello et al. 2010; Pörtner et al. 2014; Thurber et al. 2014). Despite the lack of information about the benthic community impacts of trawling in South Africa, trawling is recognised as a threat to South African deep-sea benthic habitats, yet without a good overview of species diversity in these areas it is difficult to gauge impacts in these environments (Atkinson et al. 2011a; Sink et al. 2012a). Benthic communities are used to assess environmental change as they are known to respond to a variety of pressures and changes (Fleddum et al. 2013) and thus it is important to study these communities.

Environmental DNA provides us with a tool to monitor a large component of deep-sea species diversity, thereby potentially building towards a more comprehensive biodiversity inventory compared to identification based only on taxonomy (Sinniger et al. 2016; Deiner et al. 2017). Using eDNA extracted from deep-sea sediments collected during an ongoing monitoring programme on Childs Bank (Figure 1), this project aims to *i*) determine the community composition of the study site and *ii*) to compare the differences in biodiversity between actively trawled sites and sites that are no longer trawled.

More specifically, the objectives of this study are:

- To identify taxa to the lowest possible taxonomic group and determine the community composition of the sites and lanes (trawled and untrawled) [Chapter 1]
- To compare taxa found to existing species lists of the study site [Chapter 1]
- To compare biodiversity of epifauna and infauna, using eDNA sediment samples from actively trawled sites and sites that have been closed for trawling adjacent to Childs Bank on the West Coast of South Africa [Chapter 2]

Chapter 1: Determining community composition of study sites

Introduction

The deep-sea is the largest environment on earth, covering about 63% of the planet's surface (Thurber et al. 2014). Deep-sea environments are important in terms of climate regulation, as they help absorb excess carbon dioxide and heat from the atmosphere (Levin and Le Bris 2015). In addition, deep-sea environments are also commercially important, as they provide numerous services including fisheries and mineral resources such as oil and gas (Thurber et al. 2014). Yet the deep-sea remains one of the most understudied environments on earth, with most species especially smaller, cryptic and/or fragile taxa remaining undescribed (Levin and Le Bris 2015; Sinniger et al. 2016). Not only are many species unknown, ecological information about those that have been described is limited (Mengerink et al. 2014; Taylor et al. 2017), and for many species even understanding their distributions is difficult (Baco et al. 2016). As the deep-sea is increasingly exposed to overexploitation, pollution and climate change effects such as warming, acidification and deoxygenation (Levin and Le Bris 2015; Levin et al. 2020), our lack of knowledge about them becomes a greater concern. Conservation strategies rely on knowledge of what needs to be protected, but for the deep-sea, our knowledge of the existing biodiversity and their life-history traits to inform policy making is generally lacking (Levin et al. 2020). This lack of knowledge is partly due to the remoteness of the deep-sea, which makes it logistically difficult and expensive to sample and study. According to Levin et al. (2020) research priorities for the deep-sea include the discovery and characterization of new species and collecting life-history traits and other biological data to support policy making for management and conservation.

In South Africa, there is a pressing societal need to expand our understanding of deep-sea ecosystems. Around 65% of South Africa's Exclusive Economic Zone (EEZ) is deeper than 2000m. Yet as of 2010, 83% of sampling effort came from depths less than 100m (Griffiths et al. 2010). In recent years a concentrated effort has been made to sample offshore South African invertebrate species and a number of new species have been described (Atkinson & Sink 2018). The recent publication of the *Field Guide to the Offshore Marine Invertebrates of South Africa* (Atkinson and Sink 2018) showcases these efforts. The guide contains descriptions of 409 species and classifies a number of unknown species into one of the following 12 phyla: Porifera, Cnidaria, Sipuncula, Annelida, Arthropoda, Bryozoa,

Brachiopoda, Mollusca, Cephalopoda, Echinodermata, Chordata, and Hemichordata. In the process of creating the guide, 21 new species were described (Atkinson and Sink 2018). However, the taxonomy of many marine groups, especially in the deep-sea remain outdated with most of the research based on taxonomy from the 1900s (Sink et al. 2019). In addition, many groups still lack molecular information since many genetic studies tend to focus on commercially important species (Sink et al. 2019). Recent efforts from projects such as the SeaKeys Project and the South African chapter of the Barcode of Life project, have included barcoding species in surveys, which aids in constructing vital reference barcode databases (Sink et al. 2019). This forms an important consideration for metabarcoding studies, such as those utilising environmental DNA.

Environmental DNA involves extracting DNA (both intra and extracellular) from an environmental sample such as water, sediment or soil or even air (Deiner et al. 2017). A few studies have applied this technique to deep-sea environments, as it is often more cost-effective and less time consuming than other sampling methods and resolves a greater portion of biodiversity (e.g. Thomsen et al. 2016; Sinniger et al. 2016; Everett and Park 2018). There is a wide range of studies covering numerous topics and methods. Some studies used water samples (e.g. Thomsen et al. 2016) and other used sediment samples (e.g. Sinniger et al. 2016) or even polymetallic nodules (Laroche et al. 2020) for their work. Within the context of species discovery, Sinniger et al. (2016) conducted a study to analyse deep-sea sediments from a range of deep-sea environments worldwide. Although they were able to identify several different groups of benthic metazoans, much of the diversity within a number of phyla remains unknown (Sinniger et al. 2016). Everett and Park (2018) used environmental DNA samples to study deep-sea corals and were able to separate several cryptic species. When analysing the eDNA found on polymetallic nodules, sediments and water, Laroche et al. (2020) observed that seamounts have distinct communities in these different substrates, highlighting the ability of metabarcoding techniques to discriminate between different communities within a small geographic range. All the above examples and studies serve as evidence for the use of eDNA in sampling deep-sea communities. The only major concern is the lack of barcode information for many deep-sea species (Sinniger et al. 2016) as this limits identification of organisms to higher taxonomic levels.

This chapter aims to determine the community composition of deep-sea benthic communities on Childs Bank off the West Coast of South Africa (Figures 1 and 2) using eDNA extracted from sediment samples and to compare the taxa found to existing knowledge of species in the area. This is the first study in South Africa to use environmental DNA for deep-sea sediment sampling. I expect to find high levels of biodiversity since South Africa is known to have high levels of marine biodiversity (Griffiths et al. 2010). The South African deep-sea remains understudied (Griffiths et al. 2010) and as such it is important to utilise all available methods to describe deep-sea biodiversity; as such, we expect to find taxa from groups that have not been extensively described in the South African deep-sea environments. Currie et al. (2020) highlights the value of having historical data to track changes in communities. While these data are not always available, the need to establish baseline community information is urgent, so that the effects of anthropogenic disturbance and climate change can be measured. This chapter provides new insights into previously understudied communities on Childs Bank.

Materials and Methods

The study site

The study site, Childs Bank, is an offshore submarine feature off Hondeklip Bay on the West Coast of South Africa (Sink et al. 2012a) (Figure 1). Childs Bank falls under one of the nine endangered offshore habitats, the Southern Benguela Submarine Bank (Sink et al. 2012a). This area is considered an Ecologically or Biologically Significant Marine Area (EBSA) and forms part of one of the new Marine Protected Areas (MPAs) from Operation Phakisa, as it is considered vulnerable to mining and trawling as well as being identified as an important area for supporting bycatch management and fisheries sustainability (Harris et al. 2014a).

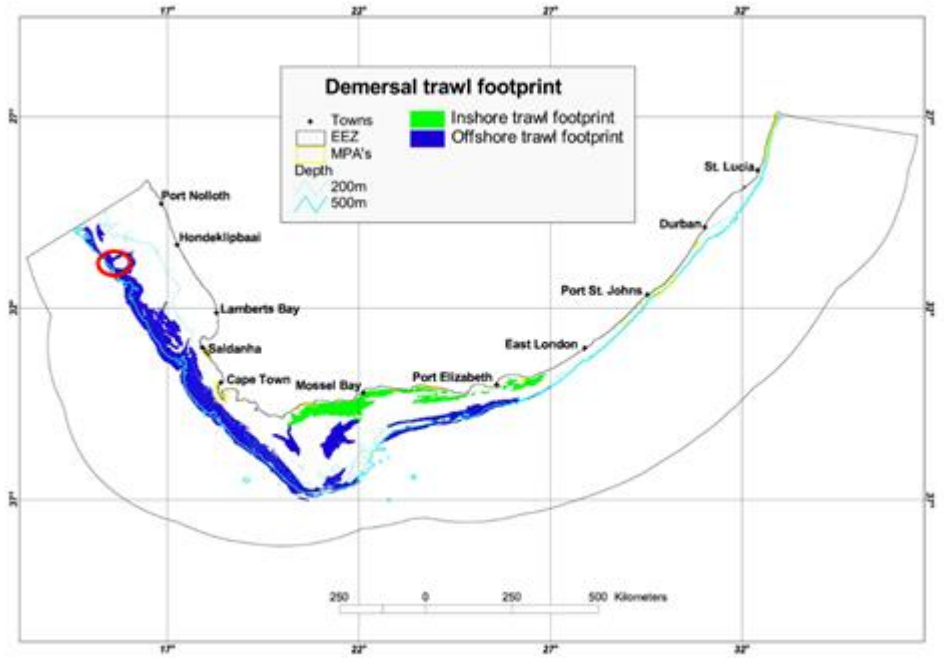

Figure 1: Map of South African trawl footprint. Red circle indicates the position of Childs Bank. Lara Atkinson, SAEON.

As part of a project with the South African Earth Observation Network (SAEON) on assessing the potential recovery of benthic habitats after trawling, three trawling lanes to the west of Childs Bank have been closed for trawling since January 2014, while two others were left open to trawling. The project's overall focus was to characterise fish and invertebrate (both epifaunal and infaunal species) diversity, as well as analysing sediment properties to determine the effects of trawling on this region. Grab samples and benthic camera images were used to identify species. In 2018, the opportunity arose to obtain some sediment from the ongoing survey for an eDNA based assessment of benthic biodiversity, which led to this project.

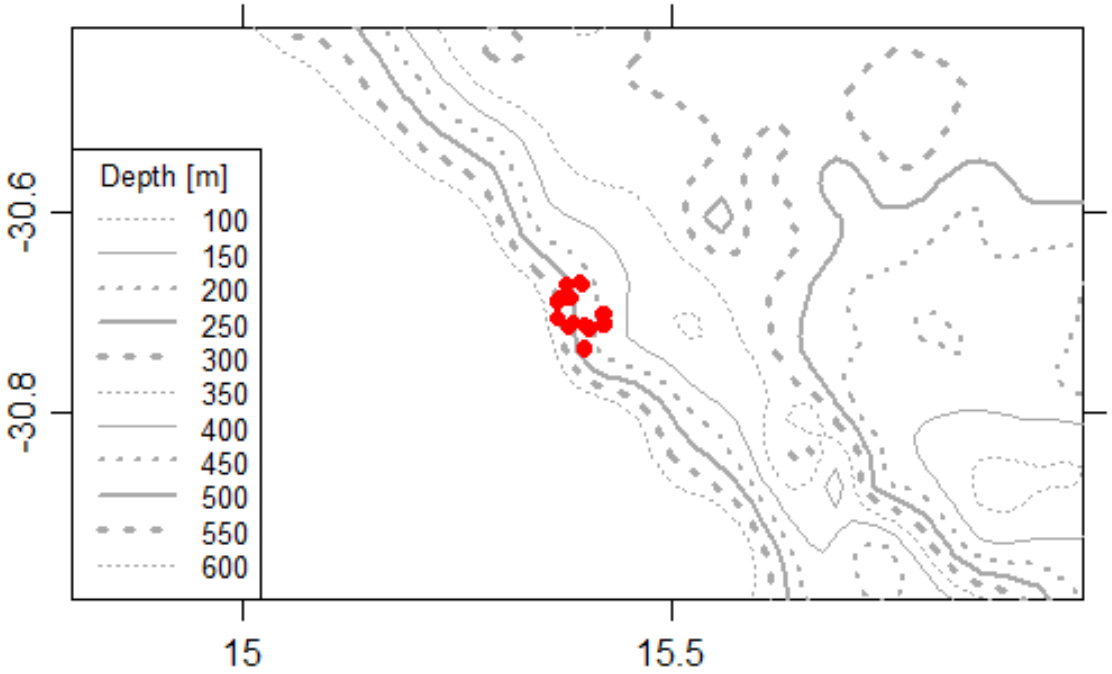

Figure 2: Close-up of study area. Area of map is the same area indicated by the red circle on the map in Figure 1. Red dots indicate triplicate grabs at each site.

Sampling regime for eDNA

In each of the lanes, both the lanes closed to trawling and the lanes open to being trawled, three stations were arbitrarily selected for sampling. Three replicate grabs were taken at each station, using a Van Veen grab sampler (0.1 m^2 can be sampled up to 20 cm deep) lowered down to the seafloor to scoop up the sediment. In total, there were fifteen sampling stations, three in each of the five trawling lanes but at the fourth lane, only two stations were sampled. Three replicate grabs were taken at each station (Figure 2), giving a total of 44 sediment samples that were stored in 500ml jars (one jar of sediment from each grab). The sediment for eDNA samples was collected from the grab before the contents of the grab were deposited on deck to minimise contamination. Samples were frozen immediately after collection at -20ºC and transported to Stellenbosch University, where they remained frozen until DNA extraction.

	X		X	
X		X		
				X
			X	
X	X			X
		X		
X				
			X	
	X			
		X		X
Closed	Open	Closed	Open	Closed

Figure 3: Figure showing experimental setup with five trawl lanes, with 2 open (white) and 3 closed (grey). Within each lane, the three randomly selected stations are identified (indicated by X). At each station three replicate grabs were taken.

DNA extraction

Grabs were subsampled for DNA extraction, as it has been shown that extraction replicates improve diversity estimates, coverage of target groups and the separation of samples with differing characteristics (Lanzen et al. 2017). Three subsamples (technical replicates) were taken from each grab for extraction purposes (Figure 3). Each jar (representative of each grab) was divided into thirds. One extraction replicate was taken from each third, so that a total of 0.25g of sediment per replicate was extracted. DNA was extracted directly from the sediment samples using the DNeasy PowerSoil extraction kit (Qiagen) following the manufacturer's protocol except that a final elution volume of 25 µL instead of 50 µL as stated in the protocol was used. The final elute was then passed through the spin column a second time before storage to ensure that as much DNA as possible was recovered. This was because initial DNA concentrations for numerous samples were low (ranging between 1 and 3 ng/ µL). DNA extractions were stored at -20ºC, with a total of 29 sites extracted (Figure 3), labelled A0001 to A0029.

Library preparation

Extracted DNA from each of the selected sites were sent to the Advanced Identification Methods (AIM) lab in Germany for library preparation using a two step PCR protocol and using the COI primers from Leray et al. (2013). This primerset was chosen as it has been

used effectively in isolating metazoan taxa from gut samples (Leray et al. 2013), water samples and sediment samples (Holman et al. 2019).

Briefly, from each sample, 5 µL of extracted genomic DNA was used, along with Plant MyTAQ (Bioline, Luckenwalde, Germany), and High Throughput Sequencing (HTS) adapted mini-barcode primers (also see Morinière et al., 2016; Morinière et al., 2019 for a description of the methodology) were applied for multiplex PCR. The initial PCR reaction was as follows: 95°C for 5 minutes, 3 cycles of [96°C for 15s; 48°C for 30s; 65°C for 90s], 30 cycles of [96°C for 15s; 55°C for 30s; 65°C for 90s] and 76°C for 10 minutes. Amplification success and fragment length were then observed using gel electrophoresis. Amplified DNA was cleaned and resuspended in 50 µL pure water for each sample before proceeding. Illumina Nextera XT (Illumina Inc., San Diego, USA) indices were ligated to the samples in a second PCR reaction applying the same annealing temperature as for the first PCR reaction but with only 7 cycles. Ligation success was again confirmed by gel electrophoresis. DNA concentrations were measured using a Qubit fluorometer (Life Technologies, Carlsbad, USA), and samples were combined into 40 µL pools containing equimolar concentrations of 100 ng each. Pools were purified using MagSi-NGSprep Plus (Steinbrenner Laborsysteme GmbH) beads. A final elution volume of 20 µL was used. High-Throughput Sequencing (HTS) was performed on an Illumina MiSeq v3 (2*300bp, 600 cycles, maximum of 25 million paired-end reads) chemistry.

Bioinformatic analyses

The AIM lab carried out the initial bioinformatic processing. After sequencing, an initial quality control analysis of the reads was performed using FastQC version 0.11.8. Merging of paired-end reads was performed using usearch v11.0.667 with the parameters *-fastq_maxdiffs 99 -fastq_pctid 75 -fastq_trunctail 0*. Primers were trimmed using cutadatpt 1.18 with Python 2.7.15. In the next step, sequences were kept above a minimum length of 300, and with a maximum of 1 expected error. Of those, unique sequences and singletons were filtered for. Quality filtering was performed using vsearch 2.9.1 with the parameters *–fastq_minlen 300 –fastq_maxee 1*. To save processing power, OTU clustering was performed before detecting chimeras. Clustering was performed using vsearch 2.9.1 and the parameters *–id 0.98 –iddef 0*

–centroids. OTU cutoff was 97%. Chimeras were detected using vsearch 2.9.1 and the parameters *–uchime_denovo –nonchimeras*.

Taxonomic assignment of the OTUs generated was performed using a BLAST search. Two databases were included in the search, namely GenBank (NCBI) and the Barcode of Life Database (BOLD). The RDP Classifier (Porter and Hajibabaei 2018a) was used to assign taxonomy to OTUs from the Ribosomal Database Project (Maidak et al. 1996). Although the RDP Classifier was originally created for ribosomal genes such as 16S, Porter and Hajibabaei (2018a) recently created a COI database mined from the GenBank database that can be used with the RDP Classifier methods. OTUs that were classified as non-marine organisms were discarded. Retained OTUs were compared between each of the datasets generated from the three different databases by constructing a Venn diagram. All OTUs that were kept were manually compared to the NCBI database again using BLAST analyses to check their closest matches. All terrestrial OTUs and OTUs that could not be identified further than Domain level were discarded. The final list of OTUs was compared to both the *Field Guide to the Offshore Marine Invertebrates of South Africa* (Atkinson and Sink 2018) and the species list from the same sampling cruise obtained from physical sampling methods (grab samples and underwater camera samples). The latter was kindly provided by Dr Natasha Karenyi from the University of Cape Town.

Accumulation curves were generated based on OTU richness per site. A curve for total diversity was generated as well as separate curves for trawled and untrawled sites. Analyses were done in R version 4.0.3 (2020-10-10), using the following packages: vegan (Oksanen et al. 2020) and readxl (Wickham and Bryan 2019).

Results

Sequencing results and comparison with existing species lists

A total of 1844876 paired-end reads were obtained from sequencing. After paired-end merging, quality filtering and de-replications, a total of 24154 unique, non-singleton sequences were kept. 1975 OTUs were retained after chimeras were discarded. After taxonomic annotation, a total of 386 OTUs from the BOLD dataset, 444 OTUs from the

NCBI dataset and 139 OTUs from the RDP dataset were retained. The OTUs retained differed between the different datasets (Figure 4).

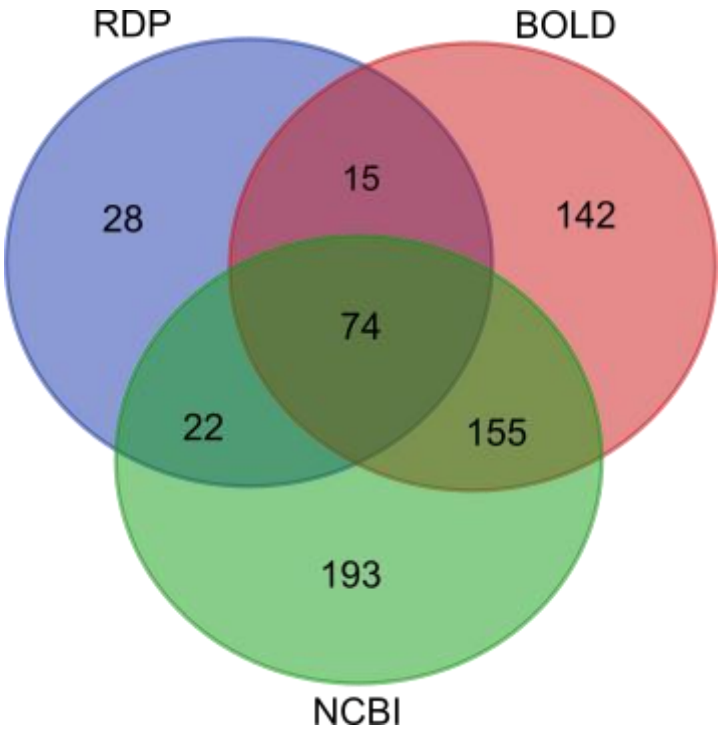

Figure 4: Number of OTUs retained for each of the three databases after taxonomic identification.

For example, the NCBI database returned higher numbers of algae and bacteria. While some of these taxa are marine, they were not used in subsequent analyses as the focus of the study was on metazoan species. The BOLD database returned a large number of insect taxa which were discarded. Certain OTUs were identified differently between databases, for example OTU_3 was identified by BOLD and RDP databases as a polychaete while the NCBI database identified it as a cephalopod; in these instances the OTU was also removed from the dataset. The NCBI and BOLD databases shared the largest number of OTUs kept (Figure 4). As the the NCBI dataset had the higher number of retained OTUs, it was selected for further analyses. After reconducting a BLAST search of the OTUs kept, 162 OTUs remained in the NCBI dataset to be used for further analyses. Of these OTUs, only 62.5% could be identified to order level (Table 1). In total, only 10 species could be recovered, 6% of OTUs (Table 1).

Table 1: Percentage of OTUs identified in each taxonomic level.

	Domain	**Phylum**	**Class**	**Order**	**Family**	**Genus**	**Species**
No. OTUs	168	168	141	105	51	21	10
Total OTUs	168	168	168	168	168	168	168
Percentage	100.00	100.00	83.93	62.50	30.36	12.50	6.0

A total of 48 unique orders were identified in the final NCBI OTU dataset but of these, only 21 were also identified by the field guide and only eight by the species list (Table 2). This pattern was repeated across family, genus and species level (Table 2).

Table 2: Comparison of numbers of taxa found between metabarcoding sample list and physical sample lists. *list kindly provided by Dr N Karenyi from University of Cape Town

	Order	**Family**	**Genus**	**Species**
Unique ID	47	41	19	10
Unique ID matched in guide	21	6	4	1
Unique ID matched in list*	8	7	2	0

The taxa found ranged over a number of different phyla (Figure 5) and were relatively evenly spread across sites, with the exception of sites A_0026, which was dominated by the orders Teuthida and Apodida and A_0015 which was dominated by unassigned OTUs (Figure 6). Sites A_0006, A_0009 and A_0018 had no OTUs that could be identified to the level of order (Figure 6).

Physical samples were collected from the same grabs as the eDNA samples and Dr. Karenyi provided a list of taxonomic identities of these physical samples. The list of physical samples taken identified a total of 88 specimens to species level from 71 genera and 55 families (Table 3).

Table 3: Numbers of unique taxa of physical specimens collected alongside metabarcoding samples. Data provided by Dr Natasha Karenyi, UCT.

	Order	Family	Genus	Species
Unique ID	28	55	71	88
Unique ID matched in eDNA	8	7	2	0

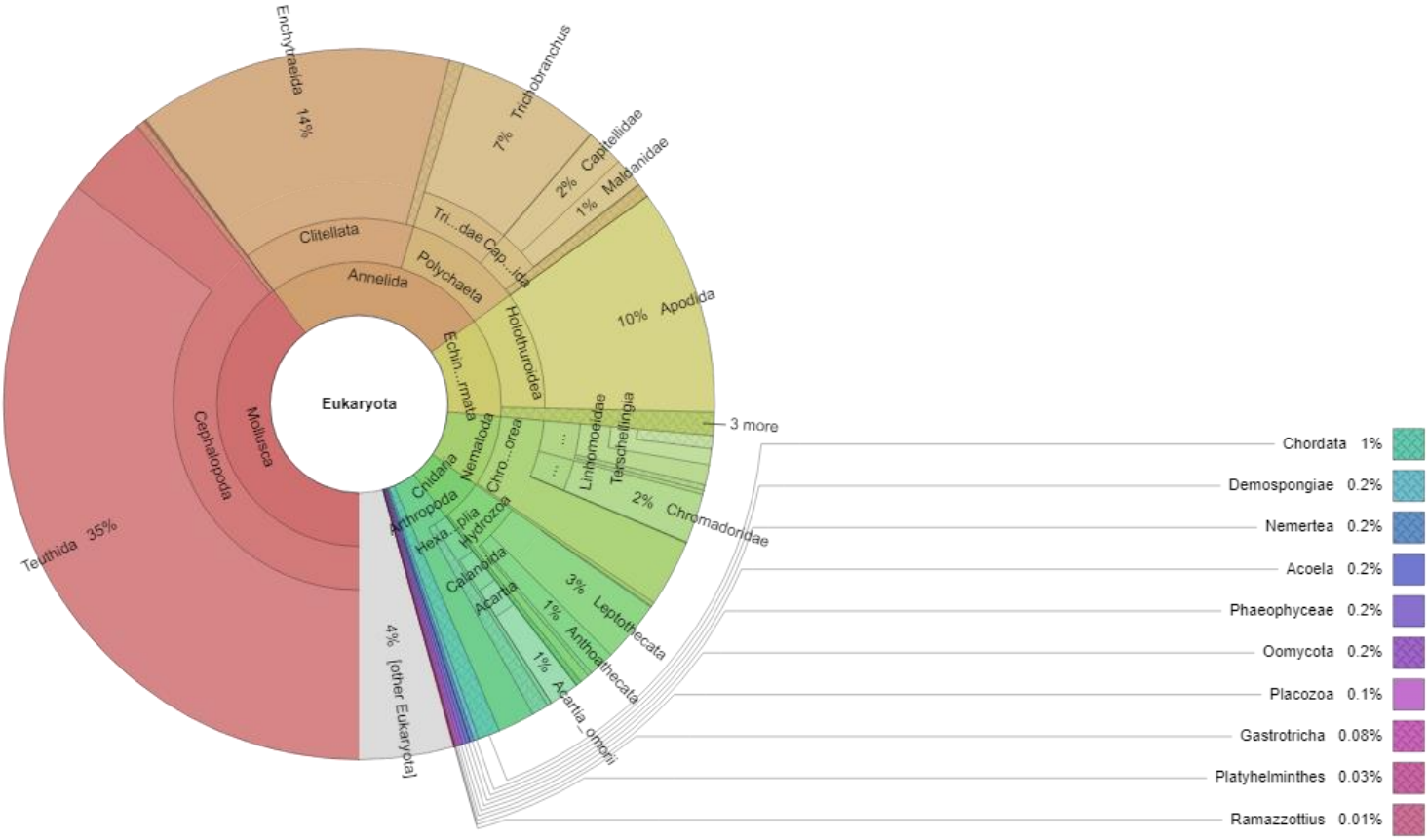

Figure 5: Krona graph showing the proportions of OTUs belonging to the different phyla identified by eDNA samples.

In order to visualise the proportion of total diversity collected, i.e. across all sites, accumulation curves were constructed. The curve for total diversity is very steep and has not reached a plateau (Figure 7) indicating a large proportion of unidentified taxa may remain.

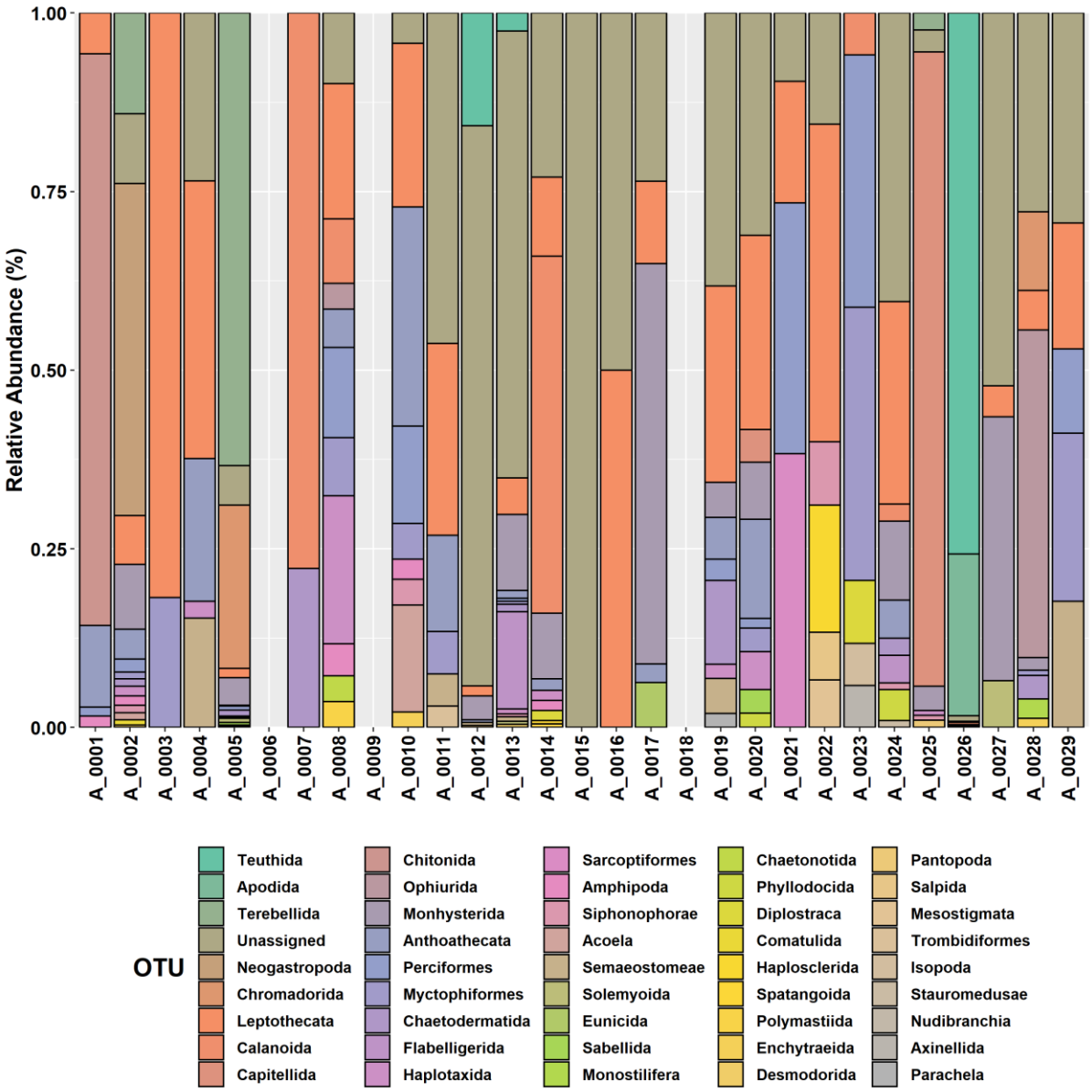

Figure 6: Relative abundances of orders found at sites A0001 – A0029.

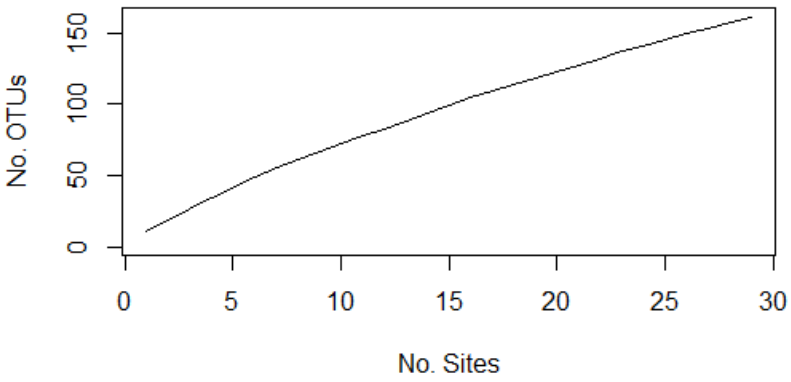

Figure 7: Accumulation curve showing the number of OTUs as a function of sampling effort, represented here by number of sites.

Discussion

This chapter represents the first attempt of utilising eDNA metabarcoding on deep-sea sediments in southern Africa. The primary aim was to identify the community composition of deep-sea sediments and to compare the OTU taxonomy to existing species inventories. As expected, a high level of deep-sea benthic diversity was recovered, much of which was previously unrecorded, covering 41 families and 19 genera and there was little overlap with species records from existing databases.

Differences between OTU recovery from three different databases

The importance of a complete reference database in metabarcoding studies has been highlighted repeatedly (Carugati et al. 2015; Sinniger et al. 2016; Stat et al. 2017). Without a good reference database, OTUs cannot be taxonomically labelled/identified. As shown in Figure 4, the three different databases used for taxonomic assignment of OTUs had varying results, both in terms of the number of OTUs retained and in actual assignments. After taxonomic assignment, the highest number of OTUs (444 OTUs) were kept from the NCBI database including a total of 193 OTUs that were not identified by either of the other two databases (Figure 4). 386 OTUs were retained from the BOLD database after taxonomic assignment with a total of 142 OTUs that were not in the other two databases (Figure 4). The question of how complete these databases are remains. Kvist et al. (2103) showed that both BOLD and NCBI databases for COI only cover ~15% of known biodiversity. Some groups in

particular e.g. the Platyhelminthes, that are known to be species rich, are highly underrepresented (Kvist et al. 2013). They emphasise that this can only be attained by an increase in barcoding efforts but that this must be underpinned by accurate taxonomy to avoid issues such as inaccurate naming or labelling of target species (Kvist et al. 2013). A more recent investigation by Porter and Hajibabei (2018b) shows that there has been an increase in the number of COI records on GenBank, but at the same time, the number of insufficiently described records have also increased. Insufficiently described records are those that lack information such as location or description or collector information (Porter and Hajibabei 2018b). They also found that COI records in the NCBI database and BOLD are not always correctly or sufficiently cross-referenced or synced, creating difficulties in re-usability of COI records (Porter and Hajibabei 2018b). Singh et al. (2021) found similar problems in their study on South African zooplankton, where COI barcodes in BOLD and GenBank were not completely described or correctly cross-referenced. They also found that there were regional gaps in barcoding efforts, where South African species were underrepresented in both the BOLD and GenBank databases (Singh et al. 2021). When comparing the OTU IDs between the NCBI and BOLD databases for this study, the same issue arose. Certain OTUs were identified as completely different organisms by the different databases. This problem creates the need for the creation of custom databases for regional biodiversity, with georeferencing and correct identification of species, as well as the continued integration of barcoding of specimens collected for taxonomic purposes (Singh et al. 2021; Czachur pers. comm. for southern African marine fishes).

Overview of taxa found and comparison with other species inventories

South African marine biodiversity is relatively well documented with around ~13,000 species described (Griffiths et al. 2010). Yet most of these species have been described from habitats shallower than 100m and biodiversity remains understudied (Griffiths et al. 2010). Recent work has led to the publishing of a field guide of marine invertebrates from deeper environments, which describes 409 species across 12 phyla (Atkinson and Sink 2018). In this study, 106 OTUs from 48 orders and 42 families were identified, representing the following phyla: Mollusca, Echinodermata, Annelida, Nematoda, Cnidaria, Arthropoda, Chordata, Chaetognatha, Xenacoelomorpha, Nemertea, Gastrotricha, Placozoa, Porifera, Platyhelminthes and Tardigrada (Figure 5). While there was overlap between some of the

phyla identified, there are phyla identified by the guide that weren't identified in the eDNA samples and vice versa (Figure 8). The same is true of the species list of physical samples collected alongside the eDNA samples. The list contains 193 samples from the following phyla: Mollusca, Echinodermata, Arthropoda, Annelida, Cnidaria, Chordata, Bryozoa, Retaria, Nematoda, Porifera and Sipuncula, of which, only 87 could be identified to species level. As expected, the overlap of taxa identified between the eDNA samples and other sampling methods decreases with the level of identification, in other words; the lower taxonomic levels such as species or genus did not have a strong overlap in terms of organisms identified between different sampling methods (Table 2). In the current study, both the field guide and the species list contained unknown species that could only broadly be classified to a certain phylum or order (see Atkinson and Sink 2018), and it is possible that some of the OTUs identified may represent some of these unknown species. Confirming this, however, is difficult, since many species in this region lack barcoding information (Sink et al. 2019), making molecular identification difficult.

While the species list of physical samples collected alongside the eDNA samples identified 87 taxa to species level, none of them are shared with the 10 species identified in the eDNA samples (Table 2). Of the 409 species identified in the field guide, only one was shared with the species identified by the eDNA samples (Table 2). It is not unusual for studies employing different sampling methods to find different taxa. For example, Thomsen et al. (2016) found small differences between the fish taxa caught in a trawl net and the fish taxa identified by eDNA samples taken from the water at the same time and place. Lejzerowicz et al. (2015) also found numerous differences between the taxa found in physical sampling and by eDNA sampling of marine sediments. Reasons for this could be that certain taxa are able to avoid physical capture equipment such as nets or grabs as well as less-invasive sampling equipment like cameras (Thomsen et al. 2016) or that some taxa are difficult to identify and could be cryptic species (e.g. Everett and Park 2018). This highlights the importance of using different sampling methods to complement one another to ensure an accurate and complete representation of the communities being sampled. This has been supported through other studies in the eDNA literature; for example, Cole et al. (2021) showed that baited remote underwater videos, in conjunction with multiple eDNA surveys best resolved community structuring of fishes. In their results on recolonisation of deep-sea vent communities, Cowart

et al. (2020) recommended that physical sampling should be combined with eDNA sampling to give a more complete picture of the recolonisation process. In a South African context, until the barcode database is significantly improved, multiple techniques, including eDNA, will likely provide the best overview of deep-sea benthic biodiversity.

Figure 8: Numbers of phyla that were shared between eDNA sample list, physical sample list and field guide.

One of the more notable groups identified through sediment eDNA metabarcoding and not by other methods, was the Tardigrada. Only one OTU represented this group and was identified from one site. Tardigrades are a group of micrometazoans, between 50 µm and 1mm in size (Bartels et al. 2016), mostly known for their resilience to extreme conditions by entering a state of cryptobiosis (Sørensen-Hygum et al. 2018). Tardigrades are an understudied group (Bartels et al. 2016) with only about 156 species known from Africa as of 2001, with no marine tardigrades described in Africa (Jörgensen 2001). In South Africa, only 37 species of tardigrade are known (SANBI) and none described from marine environments. As such, this finding will be the first record of a marine tardigrade from South Africa.

Among the taxa identified in the eDNA samples were several OTUs from the groups Nematoda and Nemertea (Figure 5), which were not included in the field guide. Five OTUs were classified as Nemertea although none could be identified beyond family level. Most nematode OTUs were only identified to family level although two OTUs could be assigned to the genus *Terschellingia*. The list of physical samples included samples of Nematoda although none were identified beyond the phylum level. In South Africa, marine nematodes remain an understudied group (Griffiths et al. 2010).

With regards to fish taxa, only a few OTUs in the eDNA samples matched with fishes, which was unexpected given that Childs Bank is a well known and utilised fishing ground. The lack of fish in the eDNA samples may well reflect the gene region of choice and it has been shown that the COI marker is not always an ideal marker for detecting fishes (Collins et al. 2019). Other studies have successfully used the 12S marker to identify fish species and investigate fish community assemblages (e.g. Miya et al. 2015; Yamamoto et al. 2017). Another reason for this finding is that fish eDNA abundance in sediment may be less in comparison to other benthic taxa that live on or in the sediment. The three fish taxa found in this study were a 100% match at species level: *Bathydraco antarctica*, *Nanobrachium achirus* and *Lycodapus pachysoma*. Species from the genus *Nanobrachium* have been recorded along the South African coastline previously (www.fishbase.org). *Bathydraco antarctica* is often recorded at depths of up to 2,000m, which is much deeper than our present sampling depth of 400m-500m (Eastman 2017). However, this species has been found at shallower depths before (Eastman 2017). In general, *Bathydraco antarctica* is found further to the south (www.fishbase.org). The eDNA samples did not contain any hake (*Merluccius capensis* and *M. paradoxus*) or kingklip (*Genypterus capensis*) DNA, species of commercial importance from this region (Atkinson 2009; Griffiths et al. 2010). As such, future eDNA surveys for commercially important fishes, may need to design species-specific primers to ascertain their presence (e.g. Thomsen et al. 2012b).

Discarding reads and gene region choice: implications for analyses

A large number of reads had to be discarded since they could not be identified accurately, for example, OTU_3 that was identified by BOLD as a polychaete while the NCBI database identified it as a cephalopod or OTU_ 6 that was classified as both a mollusc and an arachnid.

There are a few different reasons that may contribute to the uncertainty of some taxonomic assignments. The first is the lack of barcoding information for many species (Sink et al. 2019). Deep-sea species in general, not just in South Africa, often lack adequate genetic information (Sinniger et al. 2016; Taylor and Roterman 2017). In addition to this lack of barcode information, a large proportion of deep-sea species remain undescribed in general (Sinniger et al. 2016). For example, just during the compilation of the *Field Guide to the Offshore Marine Invertebrates of South Africa*, 21 new species were described (Atkinson and Sink 2018). The unassigned reads could possibly be linked to species that are not represented in barcode databases or may still be undescribed.

Gener region choice (and by implication the choice of primer set) also affects the outcome of eDNA studies. A number of different primers have been designed specifically for metabarcoding purposes. The COI marker is widely used when studying animal groups (Deagle et al. 2014). One of the major advantages of this region is that it is widely represented in barcoding databases, making identification easier (Deagle et al. 2014). COI primers have successfully been used to characterise metazoan communities from different types of eDNA samples, including in sediments (Leray et al. 2013; Wei et al. 2018; Cordier et al. 2019; Holman et al. 2019; Laroche et al. 2020). However, there are also a number of concerns surrounding the use of these primers. Deagle et al. (2014) argued that the primer binding sites are not highly conserved and that the primer variability results in unreliable amplification when a large sample size covering a broad range of taxa is involved. In a study by Laroche et al. (2020), two gene regions (18S and COI) were used to characterise deep-sea benthic communities from water, sediment and polymetallic nodule samples. The COI primerset had a higher percentage of unassigned taxa than the 18S primerset, athough the COI database is larger (Laroche et al. 2020). With regards to fish taxa, Collins et al. (2019) showed that, although COI barcodes were more represented in reference libraries, they were not as effective in specificity as mtDNA 12S primers. This causes problems in terms of reproducibility since the low specificity of the COI primers also causes the amplification of non-target DNA (Collins et al. 2019).

This study used the COI primers from Leray et al. (2013) since it has been shown to successfully amplify eDNA from deep-sea sediments and to identify benthic metazoans (e.g. Laroche et al. 2020). During my study, there had also been some difficulty in sequencing the

samples from using 18S primers, contributing to the choice of the COI primers. However, the choice of primer may have contributed to the large number of unassigned reads that had to be discarded for the reasons mentioned above. Even so, I believe that using the COI gene region was the best choice as many 18S databases are even more poorly populated than COI databases.

Conclusion

Here I show, for the first time, that eDNA sampling of deep-sea meiofauna provides a rich assessment of biodiversity. Future efforts to identify key groups will be essential for fully utilizing eDNA based environmental assessment, but this study provides a fundamental starting point for how we can utilize molecular methods to investigate previously unknown diversity in hard to reach, but vital ecosystems. Overall, this chapter provides new insights into ‘biodiversity at depth’and highlights the critical need for upscaling barcoding efforts for deep-sea benthic species, not only in southern Africa, but globally.

Chapter 2: Comparing community diversity between trawled and untrawled sites

Introduction

Commercial fisheries have been shown to have a strong effect on marine biodiversity such as depletion of stocks and decrease in biodiversity, changes in trophic interactions and simplification of food webs (see Morato et al. 2006; Ramirez-Llodra et al. 2011; Norse et al. 2012; Levin and Le Bris 2015). Trawling is the most commonly utilised method for deep-sea fishing and surveying (Benn et al. 2010), which involves dragging large nets with heavy weighted equipment across the seafloor (see Sink et al. 2012a for detailed description). Of all human activities on the seafloor, bottom trawling has had the greatest spatial impact (Benn et al. 2010), with about one fifth of the sea floor globally having been trawled at least once (Mengerink et al. 2014). The effects of trawling in the deep-sea include habitat destruction, disruption of sediment, fishery stock depletion, and disruption of nutrient cycling, all of which may lead to reduced biodiversity (Pusceddu et al. 2014). Fishing methods such as trawling not only impact target fish species, but also have a variety of other impacts on deep-sea biodiversity, including bycatch. i.e. the part of the catch that is either unused or unmanaged in fisheries (Davies et al. 2009). Bycatch species include all non-target species and individuals that do not fall under sustainable practices/undesirable categories of target species, but they are still an important component of the natural ecosystem (Lewison et al. 2004; Davies et al. 2009; Oliver et al. 2015). Bycatch species range from non-target fish that are not commercially valuable (Roberts 2002) to benthic invertebrates, like corals and sponges and other epifaunal and infaunal species (Roberts 2002; Glover and Smith 2003). The mortality rate for bycatch species from the deep-sea is high (100%), even when they are discarded (Glover and Smith 2003).

In the deep-sea, natural disturbances are not common and most often occur at small spatial scales, e.g. bioturbation punctuated by large, sometimes seasonal, events such as large storms or currents (Bluhm et al. 2001; Kaiser et al. 2002). In less stable habitats where natural disturbance is more common, communities tend to be more resilient to change, with species compositions remaining largely unaffected by low levels of disturbances (Kaiser et al. 2002). More complex and stable habitats are more severely impacted by disturbance than habitats that are regularly exposed to disturbances and longer-term community changes may take

place (Kaiser et al. 2002; Kaiser et al. 2006). In a review on disturbance in deep-sea communities, Harris (2014b) pointed out that recovery can range from a year in soft-bottomed areas to more than 10 years in reef or rocky areas. Typically, deeper areas also have longer recovery times, although information about abyssal depths is limited (Harris 2014b). Within the context of this work, human induced disturbance like trawling occurs at larger spatial scales, with higher intensity than natural disturbance events and often repeatedly. Even though trawling and other human induced change has been shown to have a strong effect on deep-sea communities, there are natural factors that also affect deep-sea species and communities and may play a role in their distribution. For example, sediment properties (such as size and composition) can have a strong effect on community composition as certain species have specific preferences to certain types of sediment or substrates (Harris 2014b). Although it is difficult to study deep-sea communities, some studies have attempted to link environmental variation with explaining patterns of biodiversity. For example, in a study examining the effects of environmental gradients on speciation Glazier and Etter (2014) found that morphologically similar populations of a mollusc that were initially thought to be a single species were separated into three genetically distinct groups separated strongly by depth.

The impacts of human activity on deep-sea biodiversity are very different from the impacts caused by natural disturbance (Kaiser et al. 2006). These impacts (and subsequent recovery) depend on a number of factors such as the specific type of trawling gear used, specific habitat properties, etc. (Kaiser et al. 2006; Atkinson 2009). In general, the recovery of many benthic ecosystems is slow, especially in systems where slow growing species that are important for structuring habitats are damaged (Bluhm et al. 2001; Cook et al. 2013; Harris 2014b). In a long-term experiment off the South American coast, an experimental area was ploughed after an initial biodiversity survey (Bluhm et al. 1995; Bluhm et al. 2001). Biodiversity surveys were then conducted immediately after the disturbance event and then after three, five and seven years (Bluhm et al. 2001). After the last survey Bluhm et al. (2001) showed that the benthic megafaunal communities were still different from pre-disturbance communities in taxonomic composition as well as in abundances, where certain hemi-sessile species had returned but overall abundances were still lower than before the disturbance. This difference, however, was smaller than the differences observed in earlier surveys (Bluhm et al. 2001).

While this particular study by Bluhm et al. (2001) was more focussed on simulating the damage done by mining, many of the effects of disturbance apply to trawling as well, such as the removal and damage to hard substrate, the removal of species and disruption of sediment. For example, when comparing the species assemblage of a trawled area and an area that has been closed to trawling for 20 years, de Juan et al. (2007) found that the area disturbed by trawling was dominated by species that are less vulnerable to disturbance, including burrowing epifaunal, infaunal species and motile scavenging species. The undisturbed area on the other hand, had higher abundances of surface infauna, sessile filter feeders and fish (de Juan et al. 2007). In other cases, non-trawled areas showed higher numbers of species as well as higher biomass compared to trawled areas (Koslow et al. 2001; Cook et al. 2013).

The physical damage that is caused by trawling is often associated with hard substrate environments, such as coral, where the habitat forming elements are physically broken or damaged, impacting species that are associated with these habitats (Ramirez-Llodra et al. 2011), but habitat destruction is not only limited to hard substrates which can be physically broken. Soft substrates are also sensitive to damage by trawling for example, Pusceddu et al. (2014) found that chronic trawling causes long term changes to soft-bottom areas. These changes include a decrease in organic carbon turnover and lower organic matter content as well as a decrease in meiofaunal diversity and abundance (Pusceddu et al. 2014). Puig et al. (2012) compared the effect of repeated trawling of soft bottomed habitats on continental slopes to erosion caused by agriculture on land. Over time the seafloor was smoothed out, sediment properties changed and habitat complexity is lost (Puig et al. 2012).

Trawling in South Africa has been an important component of fishing since the early 1900s (Currie et al. 2020). Some of the most important commercial fish species, the Cape hakes (*Merluccius capensis and M. paradoxus*) and kinglip (*Genypterus capensis*) are caught by trawling (Griffiths et al. 2010). Other species commonly caught include, monk (*Lophius vomerinus*) and angelfish (*Brama brama*), both of which are commercial bycatch species (Sink et al. 2019). Trawling for commercially exploited species, both inshore and offshore, occurs along most of the South African coastline (Sink et al. 2012a), with twenty-seven unique marine habitats identified in the South African trawl footprint (Sink et al. 2012a). Of these, nine habitats have been identified as areas of concern based on multiple criteria related to trawling extent and vulnerability and as such are priorities for management and

conservation (Sink et al. 2012a). Childs Bank, the study site, falls in one of these nine areas of concern, namely the Southern Benguela Submarine Bank (Sink et al. 2012a). More recently, Childs Bank was formally recognised as an EBSA (Ecologically or Biologically Significant Marine Area) (EBSA Portal:https://cmr.mandela.ac.za/Research-Projects/EBSA-Portal/South-Africa/Childs-Bank-and-Shelf-Edge-(Childs-Bank)). According to the National Biodiversity Assessment from 2018, parts of Childs Bank are still classified as vulnerable because of the fragile reef areas that have been damaged (Sink et al. 2019).

Few studies have attempted to assess the impacts of trawling on marine biodiversity in South Africa (Sink et al. 2012a) despite the long history of trawling in the region (Currie et al. 2020). One of the first studies investigating the effects of trawling in this area found that trawling intensity had no significant effect on infaunal species while epifaunal species showed declines in species abundance, richness and diversity (Atkinson et al. 2011a). Fleddum et al. (2013) used the same data to perform biological trait analyses and found that while both epifaunal and infaunal groups had traits that were significantly influenced by trawling intensity, epifaunal communities were more impacted than infaunal communities. When investigating long term changes in fish assemblages on the West Coast, Atkinson et al. (2011b) found that fish communities were strongly influenced by depth. Over a 24-year period, there were significant changes in the demersal fish communities but they found that not all of these changes could be explained by trawling/fishing pressure alone (Atkinson et al. 2011b). Environmental changes also led to regime shifts although the effect of these environmental conditions could also be compounded by fishing pressure (Atkinson et al. 2011b). When comparing historical survey data to a contemporary survey done using the same sites, conditions and gear, Currie et al. (2020) found that while catch abundances remained similar, the species composition of catches differed. This indicates that historically dominant taxa declined to such an extent that previously scarcer taxa could increase, changing the overall community structure of an area (Currie et al. 2020). Currie et al. (2020) also pointed out that, while it was not the case in their study, other factors such as depth and other environmental variables could also contribute to changes in community composition. The reduction in habitat complexity caused by trawling could also indirectly lead to changes in the benthic community (Currie et al. 2020). Environmental DNA has been shown to be an

effective method to study benthic communities in the deep-sea (see Sinniger et al. 2016; Everett and Park 2018; Laroche et al. 2020) although this has not been tested in South Africa.

The main aim of this chapter was to compare the community composition between trawled and untrawled sites in terms of the meiofaunal community diversity found in eDNA samples across 29 sites at Childs Bank, with the hypothesis that eDNA metabarcoding would detect significant differences in community composition between trawled and untrawled sites. The chapter also aimed to identify possible environmental variables (depth and sediment composition) which may also influence patterns of community composition, although a significant effect on community composition was not expected, given that the sampling area is relatively small and the depth range was not very great.

Methods

Sampling and molecular methods used to generate OTUs are provided in detail in chapter 1. The final OTU table was used to create a presence/absence dataset (Addendum A) for each site which was subsequently used for further analyses in this chapter. Environmental data used for analyses in this chapter were collected alongside the eDNA samples (see ch. 1) all kindly provided by Dr Lara Atkinson. Depth at each site as well as sediment composition/type was recorded (Addendum B). Depth varied from ~370m to 490m. The sediment at most sites was mostly made up of silt and clay.

All statistical analyses were performed using the Program R version 4.0.2 (Team R.C. 2020). Community richness was calculated as the total number of unique OTUs per site and was calculated to quantify the level of biodiversity observed at each site (Borcard et al. 2011). I used a linear model to test the main effects of depth and trawling and the interactive effect of depth x trawling on community richness. To describe the spatial change in community composition between sites we calculated Bray-Curtis similarity between all site pairs. A PERMANOVA test using the vegan package in R (Oksanen et al. 2020) was used to test the effects of trawling, depth and the interaction between trawling and depth using 999 iterations. Sediment was initially included as main effect in the model, however the lack of sediment information at the deeper sites made the model unbalanced, thus violating model assumptions of homoscedasticity, and was dropped from the analyses. A PERMANOVA was used to

account for the spatial autocorrelation that arises from using pairwise distance measures, such as Bray-Curtis (Borcard et al. 2011).

Results

The linear model found no significant effect of depth (Std. Error = 0.04095; p-value > 0.05), trawling (Std. Error = 33.76531; p-value > 0.05) or depth x trawling (Std. Error = 0.07647; p-value > 0.05). There was a significant effect of depth on Bray-Curtis similarity between sites ($F_{ddf, ndf}$ = 1.5999; p-value = 0.017), with non-significant effects of trawling ($F_{ddf, ndf}$ = 0.8394; p-value = 0.751) and depth x trawling interaction ($F_{ddf, ndf}$ = 0.8328; p-value = 0.767) (Table 4).

Table 4: PERMANOVA results based on Bray-Curtis similarity between sites.

	Df	**Sum of Sqs**	**R^2**	**F**	**Pr(>F)**
Trawled	1	0.3218	0.03321	0.8394	0.751
Depth	1	0.6133	0.06331	1.5999	0.017 *
Trawled:Depth	1	0.3192	0.03295	0.8328	0.767
Residual	22	8.4333	0.87053		
Total	25	9.6875	1.00000		

*Df - degrees of freedom; Sum Sq - sum of squares; R^2 – Rss/ss; * indicates statistical significance with P<0.05*

Discussion

Effects of trawling

Trawling has been found to impact benthic communities in the deep-sea in a number of different ways by causing disruption of sediment, habitat destruction, disruption of nutrient cycling and loss of biodiversity (Bluhm et al. 2001; Kaizer et al. 2002; Pusceddu et al. 2014; Harris et al. 2014b). Overall, there were no significant differences in community composition

between trawled and untrawled sites (Table 4) at Childs Bank. Although unexpected, there are several explanations that can provide insight into these results. The first is that following the beginning of the open/closed experiment, fishing pressure in the 'open' trawling lanes decreased dramatically, as it was difficult for the boats to only fish those areas (in addition to a price in boat fuel which made accessing Childs Bank expensive and transferred fishing pressure further south (Atkinson pers. comm.). Since fishing pressure was then technically decreased at all sites, there would not be an effect to observe after five years. Secondly, trawling may not have as great an effect on communities of certain taxonomic groups. For example, Kaiser et al. (2006) demonstrated that trawling effects and rates of recovery after trawling differ between habitat types, trawling gear types and different phyla. Although this study focussed on shallower habitats (Kaiser et al. 2006), similar patterns can be observed in the deep-sea. For example, Atkinson et al. (2011a) found that infaunal and epifaunal communities responded differently to trawling pressures; Fleddum et al. (2013) carried out a biological traits analysis on benthic communities that had been exposed to different trawling intensities along the West Coast of Southern Africa and found that epifaunal groups were generally more impacted than infaunal groups. Nematodes for example seem particularly affected by trawling, with those in heavily impacted areas having significantly lower species diversity (Pusceddu et al. 2014). For this study, most OTUs could only be identified to higher taxonomic levels which meant that I was unable to get trait information and to carry out more detailed species assemblage analyses.

Environmental effects on community composition

Benthic community composition in the deep-sea has been shown to be influenced by a variety of environmental factors such as food availability, depth, natural disturbance regimes, sediment type and organic matter (Harris 2014; Rosli et al. 2018; Wang et al. 2019). Measuring environmental variation in the deep-sea is difficult, and there is still a lot that remains unknown (Harris et al. 2014b). While the deep-sea is more homogenous than terrestrial systems, disturbance does play a role in species distribution in space and time (Harris et al. 2014b; Rosli et al. 2018). In this study, I found that depth had a significant effect on community composition, but not sediment composition. In the Yap Trench in the Western Pacific Ocean, the distribution of meiofaunal communities was also influenced by depth and sediment grain size as well as sediment type and factors relating to food

availability (Wang et al. 2019). Of the two factors in this example, sediment grain size was the strongest predictor of benthic community. Depth had a strong positive correlation to organic matter content in this case, which may be the reason for its effect on communities (Wang et al. 2019). Along the West Coast of South Africa, Atkinson et al. (2011b) found that depth played a role in the structuring of demersal fish assemblages with fish assemblages showing a distinct difference between 300-400m along the shelf-break. In their review on meiofaunal distribution patterns in the deep-sea, Rosli et al. (2018) found that meiofaunal communities vary in abundance and diversity across a range of different scales, both spatial and temporal. They also pointed out that various abiotic factors influenced food availability and that this then makes these factors a predictor for species distribution (Rosli et al. 2018). Sediment properties including type and grain size have also been shown to have an effect on structuring communities (e.g. Wang et al. 2019). In this chapter, we did not see a significant effect of sediment type on community composition. This may be due to the fact that the sediment composition did not vary greatly between sites. Sediment data were also not available for all 29 sites and the six sites that were discarded were all deeper sites. This may also account for the non-significance of sediment composition and depth in the reduced dataset.

Conclusion

This chapter is the first study in South Africa using data from eDNA samples to examine the impacts of trawling on benthic communities in the deep-sea. The findings indicate that benthic community composition is influenced by depth in this area and that trawling intensity did not have a significant effect. Depth has been shown to influence community composition in the deep-sea, both in this area (Atkinson et al. 2011b) and elsewhere (Rosli et al. 2018; Wang et al. 2019). Trawling has often been shown to have a significant effect on the structuring of benthic communities in the deep-sea (e.g. Bluhm et al 2001; Atkinson et al. 2011a; Pusceddu et al. 2014). The fact that depth clearly affects differences in communities is a clear indication of variability in communities across different habitats along the seabed, which should be considered with regards to management strategies and policies.

Chapter 3: Overall Conclusion - lessons for future eDNA metabarcoding studies for deep-sea environments in southern Africa

The aim of this thesis was to use environmental DNA samples from deep-sea sediment to broadly examine benthic communities on Childs Bank on the West Coast of South Africa. The first chapter identified 186 operational taxonomic units (OTUs) to identify different taxa from the 29 study sites. Unfortunately, many OTUs could only be identified to higher taxonomic levels such as order since barcode information was lacking for those taxa. Taxonomic assignments from OTUs were then compared to taxa previously identified with morphological sampling (both a field guide and species list). While there was an overlap between the taxa found in all three lists, there were also numerous taxa that were found in the eDNA samples that were not in the other two lists and vice versa (Tables 2 and 3; Figure 7). This shows that eDNA is a valuable tool to use in combination with other sampling methods in order to get an accurate representation of the taxa present in an area and whilst the lack of barcodes prevents more solid taxonomic assignments, is probably the best way to gather data on deep-sea biodiversity. While this study was based on the COI marker, there are studies that have had success in describing benthic communities from sediment samples using 18S markers (e.g. Sinniger et al. 2016). I would recommend that more than one marker be used in order to maximise the number of taxa that can be identified.

The second chapter used the OTU table and two environmental data sets, sediment composition and depth, gathered from the study sites to compare community composition (beta diversity) between sites in order to test for factors that may drive community composition. Due to limited data for certain sites, only trawling intensity (as either trawled or untrawled) and depth were used in the final analyses. Trawling did not have a significant effect on community composition although depth did (Table 4). In this chapter, we show that eDNA is a useful tool to examine patterns of community composition in deep-sea benthic communities. This chapter also highlights the need to collect environmental data alongside community samples (physical or eDNA samples) in order to examine patterns and drivers of community composition in the deep-sea. Better knowledge of the factors that shape deep-sea communities will enable studies to better inform management decisions surrounding the use of the deep-sea.

Environmental DNA metabarcoding is a novel field in South Africa, although a handful of studies exist. For example, Czachur et al. (2021) surveyed the entire South African coastline, using eDNA metabarcoding from water samples in order to characterise South African coastal fish diversity. They found strong patterns related to environment and seasonality (Czachur et al. 2021). Singh et al. (2021) focussed on the metabarcoding of South African zooplankton and highlighted the absence of many South African species in global barcoding databases. Holman et al. (2021) used a combination of physical sampling and metabarcoding to examine the spread of non-native marine species. This study is the first in South Africa to use eDNA to examine deep-sea benthic communities and is among a handful of studies providing insights to similar systems globally (see Sinniger et al. 2016; Laroche et al. 2020). This work is important because data on community composition and with time, with repeated sampling, biomonitoring, will provide data on how to manage and protect deep-sea biodiversity. From our findings, it is clear that eDNA has the potential to improve our knowledge of benthic communities in the deep-sea.

One of the most important considerations for future eDNA studies is the aspect of reference barcodes. Singh et al. (2021) highlighted the poor representation of many South African taxa in global databases such as BOLD and NCBI for the COI barcode. Many deep-sea species have not yet been barcoded and as a result taxonomic identification for many species is limited to higher taxonomic levels such as family or order (Sinniger et al. 2016; Taylor and Roterman 2017; Laroche et al. 2020). This obviously limits the extent to which studies can study benthic communities. Where possible, physical samples should be collected alongside eDNA samples to curate a barcode reference database. Another important aspect to consider is the environmental data that are collected from the study site. A number of different environmental factors such as sediment grain size, disturbance (natural and man-made), depth and food availability have been shown to have direct or indirect effects on benthic community composition (Harris et al. 2014b; Rosli et al. 2018; Wang et al. 2019). If environmental data such as depth or sediment properties are collected from each site, a clearer picture of all the patterns of community composition could be created. The same can be said for repeated sampling of the same sites over different time periods. Community composition and diversity can then be compared over time and changes can be observed, i.e.,

there is a need for continuous biomonitoring. Environmental DNA is a useful tool for biomonitoring as it gives an accurate snapshot of a community within a certain time frame.

As a first trial of eDNA in deep-sea sediments in South Africa, this study has successfully shown that this technique is a valuable tool to add to biomonitoring studies in the deep-sea. It can successfully identify taxa making up these benthic communities and can be used in conjunction with other environmental data to explore drivers and patterns of benthic community composition in the deep-sea.

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Addendum A: OTU presence and absence data

Table 1:

	Site												
	A_0001	A_0002	A_0003	A_0004	A_0005	A_0006	A_0007	A_0008	A_0009	A_0010	A_0011	A_0012	A_0013
OTU_3	0	0	0	0	0	0	0	0	0	0	0	1	1
OTU_5	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_13	0	1	0	0	1	0	0	0	0	0	0	0	0
OTU_26	0	0	0	0	0	0	0	0	0	0	0	1	1
OTU_22	0	1	0	0	0	0	0	0	0	0	0	0	0
OTU_30	0	0	0	0	1	0	0	0	0	0	0	0	0
OTU_120	0	1	1	1	1	0	0	1	0	1	1	1	1
OTU_104	0	1	0	0	1	0	0	0	0	0	1	1	1
OTU_42	0	0	0	0	0	0	1	1	0	0	0	0	0
OTU_34	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_37	1	0	0	0	0	0	0	0	0	0	0	0	0
OTU_35	0	0	0	0	0	0	0	0	0	0	0	0	1
OTU_44	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_54	0	1	0	0	1	0	0	0	0	0	0	0	0
OTU_133	0	1	0	0	1	0	0	0	0	0	0	0	1
OTU_58	0	0	0	0	0	0	0	0	0	0	0	1	1
OTU_73	0	0	0	0	0	0	0	0	0	0	0	1	0
OTU_216	0	1	0	1	0	0	0	1	0	1	0	0	0
OTU_309	0	1	0	0	1	0	0	1	0	1	0	0	1
OTU_82	0	0	0	0	0	0	0	0	0	0	0	1	0
OTU_91	0	1	0	0	0	0	0	0	0	0	0	1	0
OTU_601	0	1	1	0	0	0	0	1	0	1	1	0	1
OTU_533	0	1	0	0	1	0	1	0	0	0	0	0	1

OTU_86	0	0	0	0	0	0	0	0	0	0	0	0	1
OTU_94	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_118	1	0	0	0	0	0	0	0	0	0	1	0	0
OTU_171	0	1	0	0	0	0	0	0	0	0	0	0	0
OTU_600	1	1	0	0	0	0	0	0	0	1	0	0	0
OTU_201	0	1	0	1	0	0	0	0	0	0	1	1	0
OTU_130	0	0	0	0	1	0	0	0	0	0	0	1	0
OTU_214	0	1	0	0	0	0	0	1	0	0	0	0	1
OTU_105	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_122	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_135	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_257	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_302	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_168	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_457	1	0	0	0	0	0	0	0	0	1	0	0	1
OTU_197	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_384	1	0	0	0	0	0	0	1	0	0	0	0	0
OTU_400	0	1	0	0	0	0	0	0	0	0	0	0	1
OTU_462	0	1	0	0	1	0	0	0	0	0	0	0	0
OTU_206	0	0	0	0	0	0	0	0	0	1	0	0	0
OTU_608	0	0	0	1	0	0	0	0	0	0	0	0	0
OTU_486	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_279	0	1	0	0	0	0	0	0	0	0	0	0	0
OTU_914	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_270	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_709	0	0	0	0	1	0	0	0	0	0	0	0	1
OTU_604	0	1	0	0	0	0	0	0	0	0	0	0	0

OTU_954	0	1	0	0	0	0	0	0	0	0	0	0	0
OTU_875	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_459	0	1	0	0	0	0	0	0	0	0	0	0	0
OTU_742	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_707	0	0	0	0	1	0	0	0	0	0	0	0	0
OTU_1264	0	0	0	0	0	0	0	0	0	0	1	1	0
OTU_388	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_777	0	0	0	0	1	0	0	0	0	0	0	0	0
OTU_1556	0	0	0	0	0	0	0	1	0	0	0	0	0
OTU_744	0	0	0	0	0	0	0	0	0	0	0	1	0
OTU_684	0	1	0	0	0	0	0	0	0	0	0	0	0
OTU_610	0	0	0	1	0	0	0	0	0	0	0	0	0
OTU_660	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_648	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1061	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_512	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_680	0	1	0	0	0	0	0	0	0	0	0	0	0
OTU_632	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_465	0	1	0	0	0	0	0	0	0	0	0	0	0
OTU_746	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_523	0	1	0	0	0	0	0	0	0	0	0	0	0
OTU_1047	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1402	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1092	0	0	0	0	0	0	0	0	0	0	0	0	1
OTU_826	0	0	0	0	1	0	0	0	0	1	0	0	0
OTU_788	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_558	0	0	0	0	0	0	0	0	0	0	0	0	0

OTU_703	0	0	0	1	0	0	0	0	0	0	0	0	0
OTU_901	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_889	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_609	0	0	0	1	0	0	0	0	0	0	0	0	0
OTU_631	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_664	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_771	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_975	0	0	0	0	1	0	0	0	0	0	0	0	0
OTU_976	0	0	0	0	1	0	0	0	0	0	0	0	0
OTU_1147	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_994	0	0	0	0	0	0	0	1	0	0	0	0	0
OTU_730	0	0	0	0	0	0	0	0	0	0	0	1	0
OTU_1351	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_921	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_935	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_841	0	0	0	0	0	0	0	0	0	0	0	0	1
OTU_1394	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1376	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1430	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_821	0	0	0	0	0	0	0	1	0	0	0	0	0
OTU_1216	0	0	0	0	1	0	0	0	0	0	0	0	0
OTU_878	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1399	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_845	0	0	0	0	0	0	0	0	0	0	0	0	1
OTU_1567	0	0	0	0	0	0	0	0	0	1	0	0	0
OTU_1495	0	0	0	1	0	0	0	0	0	0	0	0	0
OTU_1094	0	0	0	0	0	0	0	0	0	0	0	0	0

OTU_1106	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1732	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1029	0	0	0	0	0	0	0	0	0	0	0	0	1
OTU_990	0	0	0	0	0	0	0	1	0	0	0	0	0
OTU_963	0	1	0	0	0	0	0	0	0	0	0	0	0
OTU_996	0	0	0	0	0	0	0	1	0	0	0	0	0
OTU_950	0	1	0	0	0	0	0	0	0	0	0	0	0
OTU_973	0	0	0	0	1	0	0	0	0	0	0	0	0
OTU_1023	0	0	0	0	0	0	0	0	0	0	0	1	0
OTU_1242	0	0	0	0	0	0	0	0	0	1	0	0	0
OTU_1353	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1262	0	0	0	0	0	0	0	0	0	0	0	1	0
OTU_1410	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1377	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1433	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1314	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1214	0	0	0	0	1	0	0	0	0	0	0	0	0
OTU_1162	1	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1247	0	0	0	0	0	0	0	0	0	0	1	0	0
OTU_1297	0	0	0	0	0	0	0	0	0	0	0	0	1
OTU_1280	0	0	0	0	0	0	0	0	0	0	0	0	1
OTU_1245	0	0	0	0	0	0	0	0	0	0	1	0	0
OTU_1320	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1431	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1167	0	1	0	0	0	0	0	0	0	0	0	0	0
OTU_1288	0	0	0	0	0	0	0	0	0	0	0	0	1
OTU_1849	0	0	0	0	0	0	0	0	0	0	0	0	0

OTU_1869	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1493	0	0	0	1	0	0	0	0	0	0	0	0	0
OTU_1697	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1844	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1664	0	0	0	0	0	0	0	0	0	0	0	0	1
OTU_1591	0	0	0	0	0	0	0	0	0	0	1	0	0
OTU_1810	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1814	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1770	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1499	0	0	0	0	1	0	0	0	0	0	0	0	0
OTU_1791	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1889	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1824	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1846	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1570	0	0	0	0	0	0	0	0	0	1	0	0	0
OTU_1510	0	0	0	0	1	0	0	0	0	0	0	0	0
OTU_1836	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1714	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1515	0	0	0	0	1	0	0	0	0	0	0	0	0
OTU_1900	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1632	0	0	0	0	0	0	0	0	0	0	0	0	1
OTU_1602	0	0	0	0	0	0	0	0	0	0	0	1	0
OTU_1569	0	0	0	0	0	0	0	0	0	1	0	0	0
OTU_1818	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1571	0	0	0	0	0	0	0	0	0	1	0	0	0
OTU_1919	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1557	0	0	0	0	0	0	0	1	0	0	0	0	0

OTU_1775	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1505	0	0	0	0	1	0	0	0	0	0	0	0	0
OTU_1749	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1906	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 2:

	A_0015	A_0016	A_0017	A_0018	A_0019	A_0020	A_0021	A_0022	A_0023	A_0024	A_0025	A_0026	A_0027	A_0028	A_0029
OTU_3	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
OTU_5	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
OTU_13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_26	0	0	1	0	0	1	0	0	0	1	0	0	0	1	0
OTU_22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_30	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
OTU_120	0	1	1	0	1	1	1	1	0	1	0	1	0	1	1
OTU_104	1	0	1	0	1	1	0	1	0	1	1	0	1	1	1
OTU_42	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0
OTU_34	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
OTU_37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_44	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
OTU_54	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0
OTU_133	0	0	1	0	0	1	0	0	0	0	0	0	1	1	0
OTU_58	0	0	1	0	0	0	0	0	0	0	1	0	0	1	0
OTU_73	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
OTU_216	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
OTU_309	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0
OTU_82	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_91	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
OTU_601	0	0	0	0	0	1	0	0	1	0	0	1	0	1	1
OTU_533	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0
OTU_86	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_94	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_118	0	0	0	0	1	1	0	0	0	1	0	0	0	0	0

OTU_171	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
OTU_600	0	0	0	0	1	1	0	0	1	0	0	0	0	0	1
OTU_201	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0
OTU_130	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_214	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
OTU_105	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_122	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
OTU_135	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
OTU_257	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
OTU_302	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0
OTU_168	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
OTU_457	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_197	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
OTU_384	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
OTU_400	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
OTU_462	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
OTU_206	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_608	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
OTU_486	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0
OTU_279	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_914	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
OTU_270	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
OTU_709	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
OTU_604	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_954	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
OTU_875	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
OTU_459	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

OTU_742	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_707	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
OTU_1264	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
OTU_388	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
OTU_777	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
OTU_1556	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
OTU_744	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
OTU_684	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_610	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
OTU_660	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
OTU_648	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
OTU_1061	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
OTU_512	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
OTU_680	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_632	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_465	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_746	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
OTU_523	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1047	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
OTU_1402	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
OTU_1092	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
OTU_826	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_788	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
OTU_558	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
OTU_703	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_901	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
OTU_889	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0

OTU_609	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_631	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_664	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
OTU_771	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
OTU_975	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_976	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1147	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
OTU_994	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_730	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1351	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0
OTU_921	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
OTU_935	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
OTU_841	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1394	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
OTU_1376	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
OTU_1430	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
OTU_821	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1216	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
OTU_878	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1399	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
OTU_845	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1567	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1495	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1094	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
OTU_1106	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
OTU_1732	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0
OTU_1029	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

OTU_990	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_963	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_996	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_950	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_973	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1023	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1242	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1353	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
OTU_1262	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1410	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
OTU_1377	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
OTU_1433	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
OTU_1314	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1214	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1162	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1247	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1297	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1280	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1245	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1320	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1431	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
OTU_1167	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1288	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1849	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
OTU_1869	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
OTU_1493	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1697	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

OTU_1844	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
OTU_1664	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1591	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1810	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
OTU_1814	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
OTU_1770	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
OTU_1499	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1791	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
OTU_1889	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
OTU_1824	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
OTU_1846	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
OTU_1570	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1510	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1836	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
OTU_1714	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1515	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1900	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
OTU_1632	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1602	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1569	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1818	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
OTU_1571	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1919	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
OTU_1557	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1775	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
OTU_1505	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU_1749	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0

OTU_1906	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0

Addendum B: Environmental data collected per site

				Sediment composition (%)			
Site	**Depth**	**Lane**	**Trawled**	**Gravel**	**Sand**	**Silt**	**Clay**
A_0001	373	5	N	0,091418	89,49873	8,053132	2,356723
A_0002	477	1	N	0	81,00402	14,84529	4,150691
A_0003	373	5	N	0,091418	89,49873	8,053132	2,356723
A_0004	373	5	N	0,091418	89,49873	8,053132	2,356723
A_0007	443	2	Y	0,04559	84,77519	11,64381	3,535406
A_0008	373	5	N	0,032804	94,07185	4,62839	1,266959
A_0010	414	3	N	0	87,27631	9,935384	2,788307
A_0012	447	2	Y	0,04559	84,77519	11,64381	3,535406
A_0013	478	1	N	0	81,00402	14,84529	4,150691
A_0014	373	5	N	0,032804	94,07185	4,62839	1,266959
A_0015	416	3	N	0	87,27631	9,935384	2,788307
A_0016	487	1	N	0,022541	78,50433	16,13251	5,340614
A_0020	482	1	N	0	81,00402	14,84529	4,150691
A_0021	402	4	Y	0,008251	89,84287	7,142094	3,006791
A_0022	380	4	Y	0,171773	88,56616	8,697842	2,564221
A_0023	446	2	Y	0,04559	84,77519	11,64381	3,535406
A_0024	488	1	N	0,022541	78,50433	16,13251	5,340614
A_0025	490	1	N	0,022541	78,50433	16,13251	5,340614
A_0026	402	4	Y	0,008251	89,84287	7,142094	3,006791
A_0028	415	3	N	0	87,27631	9,935384	2,788307